The impact of neonatal nutrition on the health, welfare and productivity of Holstein dairy calves

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by

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Abstract

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Dairy calves in the U.K. are currently reared on 'least cost' principles, with minimal milk feeding and early weaning to solid foods. This has been described as maintaining the calf in 'a state of chronic hunger'. As well as repercussions on calf health, growth and welfare, human studies suggest that underfeeding the newborn is a major risk factor for metabolic disease in the adult. The aims of this study were to determine current dairy calf rearing practices across the U.K., to investigate the performance of Holstein heifer calves fed increased milk replacer (MR) compared to restricted volumes, and to determine the impact of this on key performance indicators (KPIs) of these animals as calves and growing heifers.

A postal questionnaire was offered to one thousand U.K. dairy farmers to determine current calf rearing practices. The response rate was 72% and revealed that housing and feeding practices were variable between farms. The majority of farmers (93%) fed restricted volumes of milk or milk replacer to their pre-weaned calves.

The body weight, withers and loin height, heart and belly girth, crown to rump length, hock-fetlock length and body condition score (BCS) were recorded weekly from birth to 12 weeks and monthly from 12 weeks until conception in two groups of Holstein heifer calves on one commercial dairy farm in the north-west of England, U.K. Calves were assigned to a restricted, Group R (*n* = 50) or *ad libitum*, Group A (*n* = 50) MR feeding strategy from birth until weaning. Growth rates were greater for Group A (0.72kg/day) from birth until 3 weeks than Group R (0.17kg/day). Body condition score increased for Group A during this period (0.1 points) while it decreased for Group R (0.3 points). Thereafter, growth rates were similar between dietary groups although no catch-up growth was observed for Group R animals. Changes in morphometric measures were greater for Group A calves than Group R from birth to 12 weeks. From 12 weeks of age onwards, dietary group differences in morphometric measures disappeared but body weight differences remained until conception.

The glucose metabolism and insulin sensitivity of a subset of heifer calves (*n* = 6 Group A, *n* = 6 Group R) was investigated at 3, 12 and 39 weeks of age and was shown not to be affected by dietary group. The carcass composition of Holstein bull calves assigned to one of the two dietary groups was assessed. Calves were studied at birth (*n* = 3), 3 weeks, 9 weeks or 12 weeks (*n* = 3 per dietary group at each age). Carcass composition was assessed using spiral CT technologies. Group A calves had greater internal adipose deposition at all ages but there was no difference in carcass associated adipose tissue.

The age at puberty, first service and conception was between 2 and 3 weeks lower for Group A animals than for Group R. Increased MR feeding of Holstein heifers allows for greater growth rates and earlier entry into the milking herd.

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General Introduction

1.1 Welfare of food producing cattle

Evidence has shown that the domestication of cattle took place as early as around 3000 B.C. (Bollongino et al., 2006; Howard, 1961) with animals being reared for milk and meat as today. Through continued population growth and lifestyle change, the intensity in which cattle are reared and farmed over the past century has increased greatly (VandeHaar and St-Pierre, 2006).

The world population has increased from around 2 Billion in 1920 to nearly 7 Billion today (U.S., 2012). Changes in lifestyle and diet have led to a vast increase in consumption of dairy based foods globally (Halton et al., 2006). The demand for dairy products will continue to increase as more countries adopt a modern western lifestyle and buying behaviour (Boettcher, 2001).

The welfare of food producing animals is now highly important to the consumer on a worldwide scale (Vasseur et al., 2010; Ventura et al., 2015). This has catalysed a move towards higher farm animal welfare standards including free range and organic products (Farming, 2011). Practical strategies for improving animal welfare at farm level are needed to not only ensure customer satisfaction but to benefit farmed animals in the U.K. and Worldwide. Increased consumer awareness of animal welfare and food safety (origin of food, drug and antibiotic usage etc.), is a driver for research in the field of animal welfare and sustainable production. Additionally, full traceability of food products is becoming of key importance globally as demonstrated by the horse meat food scandal of 2013 in which horsemeat was being included in processed foods labelled as beef (BBC, 2013).

1.2 Dairy Herds

Over the last 3 decades there has been a trend worldwide towards reduced dairy cattle numbers (Boettcher, 2001). In 1980 there were 3.2 million dairy animals in the U.K. Since then, numbers have decreased significantly to only 1.8 million in 2013 (DairyCo., 2013). Although the numbers of dairy cows have decreased, the volume of milk produced per animal per annum has increased, from 5398 litres in 1995 to 7717 litres in 2014 (DEFRA, 2014) . This increase in yield has been attributed to a number of factors such as improved nutrition, management and genetic selection (Lucy, 2001). The drive towards higher milk

yield in individual animals has occurred as a result of both falling farm-gate prices for milk and increased consumer demand (DairyCo., 2013).

In addition to increased milk yield per cow, there has been an increase in herd size and decrease in herd numbers. In England in 1999, there were just over 28,000 dairy producers with an average herd size of 85, by 2010 there were just over 11,000 producers with an average herd size of 105 (Hawkins, 2011). This trend continues with many U.K. dairy farms today operating with over 2,000 milking animals. Unfortunately, at the other end of the scale, many small dairy herds have ceased to exist due largely to falling milk prices and increased production costs.

Historically, U.K. dairy farmers utilised breeds such as the Dairy Shorthorn or British Friesian for milk production. These breeds, although well adapted to thrive in the U.K. environment, produce relatively low yields of milk. In order to maintain profitability within a volatile market place, most dairy farmers have moved towards using breeds of cow with the genetic potential to produce significantly greater volumes of milk. The Holstein Friesian is the predominant dairy breed constituting 90% of animals in the UK dairy industry today (Roy, 1990, Dillon, et al. 2003).

Farm management has shifted from extensive pasture based methods that were seen in the 1940's, to the high input: high output systems of today (Capper et al., 2009). Although this shift has enabled increased milk production by genetically capable breeds, it has not been without cost. Increases in herd size and milk production have been related to decreases in fertility (Lucy, 2001; Thatcher et al., 2006), with conception rates dropping from approximately 60% in the 1950's to around 40% in recent years (Royal and Flint, 2004). This decline in fertility has a direct impact on longevity and cost of production within the herd. Whilst declining fertility may be attributed to many inter-related proximal factors, increasing metabolic demands placed on cows particularly during early lactation, may lead to negative energy balance (NEBAL) which can be a major factor of this decline. Energy requirements for early milk production often exceed dietary energy intake, resulting in increased lipolysis and elevated peripheral concentrations of non-esterified fatty acids (NEFAs). It has been extensively reported that for high yielding dairy cows, the risk of development of metabolic disease is heightened during the first few weeks after parturition

(Bisinotto et al., 2011; Morris et al., 2009; Sinclair, 2010; Thatcher et al., 2006; Veerkamp et al., 2003). Furthermore, the elevated concentrations of NEFAs in the peripheral circulation, have detrimental effects on granulosa cell function and may reduce fertility of these animals (Bossaert et al., 2008). Poor fertility is a major limiting factor of longevity in the Holstein dairy cow (Wathes et al., 2008) and over one third of involuntary culls are associated with this (Esslemont and Kossaibati, 1997). Although capable of living for in excess of 15 years (Nowak, 1997), many Holstein dairy cows survive only 3 lactations prior to removal from the herd (Hare et al., 2006; Haworth et al., 2008).

It has become increasingly recognised that lifetime performance and longevity is crucial for ensuring profitability and sustainability of dairy enterprises; there is considerable research interest into the impact that early life management may have on this (Bach, 2011; Morrison et al., 2012; Soberon and Van Amburgh, 2013; Van Amburgh et al., 2011; Wynn et al., 2009).

1.3 Dairy calves

Calf morbidity and mortality are of great economic importance and estimates of dairy calf mortality in the U.K. are reported to be around 8% (Brickell et al., 2009b; Esslemont and Kossaibati, 1996; Mee, 2008; Ortiz-Pelaez et al., 2008). Figures in the U.S.A (USDA, 2007) and throughout most of Europe (Gulliksen et al., 2009; Raboisson et al., 2013) are similar and have not declined over the past decade (Roy, 1990); however in Sweden, lower neonatal mortality rates of 1.2-1.5% have been reported (Svensson et al., 2006). In the U.K., financial losses due to calf mortality up to 6 months of age equate to approximately £60 million per annum (DEFRA, 2003). Over 50% of neonatal calf mortality is related to diarrhoea during the first 3 weeks of life (DEFRA, 2003). Whilst a number of pathogens (Rotavirus, Coronavirus, Cryptosporidium, *E. coli, Salmonella*) are associated with calf diarrhoea (Bellinzoni et al., 1990; Roy and Ternouth, 1972), the chief risk factors for disease are deficits in management, nutrition and hygiene (DEFRA, 2003; McGuirk, 2007; Stull and Reynolds, 2008; Windeyer et al., 2014).

In natural circumstances, a calf would remain with its mother for at least 9 months after birth (Reinhardt, 2002). Obviously the dairy industry operates by removal of the calf from the dam much earlier but there is considerable variation in the length of time the calf remains with its dam between farms (Flower and Weary, 2001). 'Snatch calving' involves removal of the calf from the dam at birth. This practice is carried out in order to reduce the risk of disease transmission, primarily from adult cow faecal contamination in the calving area (Mee, 2008). An adult cow can produce between 30 and 50kg of faeces daily; exposure of a newborn calf to this will significantly increase the risk of disease (MAFF, 1991).

Other farm practices involve leaving calves with their dams for up to four days post-calving (Weary and Chua, 2000). Whilst this can reduce the labour costs associated with manual administration of colostrum, control over the volume consumed during the critical few hours of life is lost. Studies have shown that calves left with their dam for up to 4 days are at a greater risk of failure of passive transfer (FPT) of immunoglobulins than those removed early and manually administered colostrum (Besser et al., 1991; Edwards, 1982; Svensson et al., 2006).

Recently, the link between early life events and future health and productivity of many species has been studied in more depth (Feeney et al., 2014; Funston and Summers, 2013; Singh et al., 2010). It has been reported that in humans, early life malnutrition increases the risk of diseases such as diabetes and atherosclerosis in later life, as well as other problems such as reduced adult size (Lucas, 1998; Lumey et al., 2007; Pelletier and Frongillo Jr, 1995). This was exemplified by Barker's "One thousand day hypothesis", which suggested that much of the risk for future disease is acquired during the first 1000 days of a baby's life including the 9 months *in utero* (Barker, 1998; Barker and Purslove, 1998).

Although recent work has studied the association between milk replacer intake of pre weaned calves and future productivity (Van Amburgh et al., 2011; Wynn et al., 2009), more work is necessary to enable further understanding of the relationship between early life management and nutrition and lifetime health and performance in the dairy cow.

1.4 Colostrum

In the weeks prior to parturition, immunoglobulins (Ig) and other lacteal secretions accumulate in the mammary gland primarily from the serum of the dam (Guy et al., 1994). There is a peak in Ig concentration 1-3 days before calving (Godden, 2008; Weaver et al., 2000), which then declines rapidly post-calving (Godden, 2008; Kelly, 2003).

Colostrum is the first milk produced by the mother after parturition (Pakkanen and Aalto, 1997) and consists of many components. These include vitamins, minerals, leukocytes, growth factors, and immunoglobulins (Godden, 2008). The role of all colostral components is not clear, although it has long been understood that colostrum is invaluable to newborns of many species (Kuttner, 1923; Sawyer et al., 1977 ; Smith and Little, 1922). This is especially so for the bovine neonate, who is born agamma-globulinaemic and depends absolutely on the absorption of maternal Ig from colostrum across the wall of the small intestine soon after birth for passive immune protection in early life (Arthington et al., 2000; Godden, 2008; Kuttner, 1923; McGuirk, 2007; Quigley and Drewry, 1998; Weaver et al., 2000). This absorption aids the protection of calves against pathogenic organisms prior to maturation of their own immune system (Godden, 2008). These vital immunoglobulins should be consumed by the calf as soon as possible after birth (Weaver et al., 2000). Of the immunoglobulins present in bovine colostrum, IgG accounts for about 90%, IgA 5% and IgM 5% (Godden, 2008). Absorption of macromolecules such as Ig across the small intestine of the calf can only occur during the first 24 hours of life before the intestine becomes impermeable to these (Rischen, 1981). The primary Ig in bovine colostrum is IgG1 which is derived from maternal IgG1 (Godden, 2008).

The quality and quantity of colostrum consumed is also very important (Furman-Fratczak et al., 2011; Godden et al., 2009b; Morin et al., 1997). In guidelines produced by DEFRA, it is stated that all calves should consume at least 1.5 litres of colostrum as soon as possible after birth (no longer than 6 hours) (DEFRA, 2003) and should receive a further 2 - 3 feeds of the same volume of colostrum in the first 24 hours of life (DEFRA, 2003). Based on research findings, these guidelines are an under-estimate of the volumes required for successful passive transfer of Ig in Holstein dairy calves (Besser et al., 1991; Faber et al., 2005). There is evidence to suggest that colostrum consumption at the first feed should be equivalent to approximately 10% of birth weight (Godden, 2008; Weaver et al., 2000). This guideline

assumes fair to good quality colostrum and therefore ensures consumption of at least 100g of IgG (Besser et al., 1991; Godden, 2008; Radostits and Bell, 1970), equating to approximately 3 to 4 litres of colostrum.

Components of colostrum: Maternal leukocytes contained within colostrum include neutrophils, T and B lymphocytes and macrophages (Larson et al., 1980). Once colostrum is consumed by the calf, leukocytes are absorbed across the small intestine and enter the circulation, travelling to tissues and disappearing from the circulation within 36 hours (Reber et al., 2006). It is suggested that functions of maternal leukocytes include enhanced response of lymphocytes to mitogens, increased phagocytosis, increased bacteria killing ability and enhanced IgG formation (Godden, 2008).

Growth factors present in colostrum include transforming growth factor beta-2, growth hormone, insulin and insulin like growth hormones 1 and 2 (IGF-1, IGF-2) (Elfstrand et al., 2002). Although evidence suggests that IGF-1 from colostrum is not absorbed through the small intestine of the newborn calf (Hammon et al., 2000), it may have local effects such as regulation of gastro-intestinal tract development (Baumrucker et al., 1994). The roles of the other growth factors present in bovine colostrum are largely unknown (Pakkanen and Aalto, 1997).

Colostrum contains cytokines (TNF-alpha, IL6, IL1, INF-gamma etc) that are present in much higher concentrations than in milk (Kelly, 2003). These cytokines are produced and secreted in the mammary gland and have a positive influence on neonatal immunity (Hagiwara et al., 2000).

Examples of colostral components with anti-microbial properties include lactoferrin, lysozyme and lactoperoxidase(Pakkanen and Aalto, 1997). Specifically, lactoferrin binds iron and plays a role in the activation of phagocytes and immune responses (Pakkanen and Aalto, 1997; Robblee et al., 2003). Lysozyme damages bacterial cell walls and lactoperoxidase inhibits bacterial metabolism (Law and Reiter, 1977).

In addition, colostrum has a high nutritive value. It has a 4-fold increase in protein content and a two-fold increase in crude fat content compared to milk (Foley and Otterby, 1978). Energy from fat and lactose in colostrum is used for thermogenesis in neonatal calves (Godden, 2008).

1.5. Passive transfer of Immunoglobulins

Failure of passive transfer is a major risk factor for both morbidity and mortality in calves (Beam et al., 2009; Besser et al., 1991; Godden et al., 2009a; Robison et al., 1988; Weaver et al., 2000) and is defined by calf serum IgG concentrations of less than 10 mg/mL (Chigerwe et al., 2008b; Godden, 2008; Wells et al., 1996) at 48 hours of age. Serum IgG concentration may be quantified either directly by determining IgG concentration, or indirectly by determining total protein concentrations which are highly correlated with IgG concentrations (Ameri and Wilkerson, 2008).

Techniques for direct measures include:

Radial immunodiffusion (RID): This is considered the gold standard measurement (Dawes et al., 2002), commercial kits are derived from work by Mancini (Mancini et al., 1965) and Fahey (Fahey and McKelvey, 1965). Serum samples are added to wells within agarose gel plates containing specific anti-bovine IgG. During an incubation period, IgG present within the sample diffuses into the gel forming a ring. Using standard curves, IgG concentration may be determined. This test has limitations in that it is time consuming, expensive and requires the correct technical expertise to perform the test. In addition, there have been reported discrepancies between available kits (Ameri and Wilkerson, 2008).

Enzyme linked immunosorbant assay (ELISA): This test is qualitative, producing a positive (serum IgG >10 mg/ml) or negative (serum IgG <10 mg/ml) result for success of passive transfer of IgG. The test is reported to have high sensitivity and specificity (0.93 and 0.88 respectively) and is deemed an excellent tool for evaluation of FPT in neonatal calves (Dawes et al., 2002).

Turbidimetric Immunoassay (TIA): This immunoassay works in a similar way to RID in that bovine IgG interacts with anti-bovine IgG, but a liquid media is used rather than agar. The TIA method is highly correlated with the gold standard RID method (Etzel et al., 1997). Solution turbidity is measured by machine and although a spectrophotometer is required to determine results, the test produces results much faster than RID.

Techniques for indirect measures include:

Sodium Sulphite Turbidity Test (SST): This is a semi-quantitative test that uses 14, 16 and 18% sodium sulphite solutions. Addition of sodium sulphite to serum produces turbidity from selective precipitation of high molecular weight proteins including Igs (Pfeiffer and McGuire, 1977). Increasing the concentration of the sodium sulphite solution (from 14 through to 18%) induces turbidity of samples with increasingly lower concentrations of Igs. This means that if turbidity is achieved at the lowest concentration of sodium sulphite (14%), higher sample concentrations of Igs are present than if turbidity was achieved at 16 and 18% sodium sulphite solution (Weaver et al., 2000). A disadvantage of this test is that false positive results for FPT using the 16 and 18% solutions often occur (Tyler et al., 1996).

Zinc Sulphate Turbidity test (ZST): This test works on the same principal as the SST. Development of the original test which was described by McEwan *et al* (McEwan et al., 1970), allowed for a more useful on-farm test where a single solution assay with a 30 minute room-temperature incubation period could be used. However, Tyler *et al* (Tyler et al., 1996) described how the test had an inappropriately high end point, with low specificity. Thirty one percent of calves tested in the study were incorrectly classified for passive transfer of IgG. Increasing the concentration of test solution resulted in improved test performance by dramatically improving test specificity (Hudgens et al., 1996).

Serum gamma glutamyltransferase (GGT) activity: The enzyme GGT is present in colostrum as it is produced in the mammary gland and is absorbed alongside IgG by the bovine neonate. Calves that have consumed colostrum are observed to have a greater serum GGT activity than those that have not (Braun et al., 1982; Thompson and Pauli, 1981). Although an association between GGT activity and serum IgG has been reported, accurate assessment is not possible (Parish et al., 1997). The GGT activity test has limitations, however it has a use for clinically ill calves as the test is minimally affected by hydration status.

Refractometry: Light refracted from the total protein within a sample is measured using a hand held device (McBeath et al., 1971). As the greatest constituents of total protein within serum or plasma of neonatal calves are immunoglobulins, and the correlation between total protein and IgG at this time is 0.71, the test offers a quick and simple way to monitor

passive transfer of Igs on farm (Quigley, 2006). Although the test is simple to perform, centrifugation of blood samples were required in order to harvest plasma or serum (McBeath et al., 1971). However, Wallace *et al* (Wallace et al., 2006) assessed uncentrifuged and centrifuged samples and found a high correlation between the 2 types of samples (R^2 = 0.95).

The cut-off values for determination of FPT have been studied by many research groups (Calloway et al., 2002; Dawes et al., 2002; Tyler et al., 1996; Tyler et al., 1999a). Differences in test sensitivity, specificity and correct classification of calves with FPT are reported with different cut-off values. Quigley reported that total protein concentrations of less than 5.0 g/dL (Quigley, 2006) indicated FPT. Tyler *et al* (Tyler et al., 1999a) reported total protein concentrations of less than 5.5 g/dL (sensitivity: 0.93, specificity: 0.75) with correct classification of 85% of calves indicated FPT. Calloway *et al* (Calloway et al., 2002) reported a 5.2 g/dL cut-off for FPT (sensitivity 0.89 - 0.93, specificity: 0.80 - 0.84) with 86 - 87% of calves being correctly classified. Although there are differences in sensitivity, specificity and mis-classification of individual animals depending on chosen cut-off points, the refractometer may be a very useful tool for determining FPT on farm.

Success of passive transfer of IgG depends on a number of factors:
Breed of the dam: Many studies have revealed interbreed differences in colostrum quality (Tyler et al., 1999b). The immunoglobulin concentration of colostrum from Holstein dams has been reported to be lower than that of Ayrshire, Brown Swiss, Jersey and Guernsey dams (Muller and Ellinger, 1981); in another study colostrum from Holstein cows had a lower IgG concentration than that of Guernsey cows (Tyler et al., 1999b). These differences may be attributed to genetics or to dilution effects in the higher yielding Holstein cow (Guy et al., 1994).

Dam parity: Previous work has suggested that colostrum quality of primiparous dams is lower than that of their multiparous counterparts (Morin et al., 1997; Muller and Ellinger, 1981; Tyler et al., 1999b). There are great differences in colostrum quality between individual animals (Godden, 2008; Maunsell et al., 1999). While these differences are not

associated with dam parity *per se* (Chigerwe et al., 2008a; Godden, 2008; Kehoe et al., 2011), some studies have found increased IgG concentrations in colostrum from cows of third parity and above (Gulliksen et al., 2009; Liu et al., 2009). Based on the evidence from these studies, regardless of parity, colostrum quality should be tested prior to feeding and not be automatically discarded based on parity alone.

Nutrition of the dam: There is no evidence to support the hypothesis that pre-parturient nutrition has an effect on colostral IgG concentration (Blecha et al., 1981; Quigley and Drewry, 1998) except under conditions of extreme under-nutrition. It is generally advised that producers should ensure dry cows and heifers are fed according to their requirements as stated by the NRC (Subcommittee on Dairy Cattle Nutrition, 2001).

Time to colostrum collection: The dam should be milked as soon as possible after parturition to enable the harvest of the highest possible quality of colostrum. In a study carried out by Morin *et al,* for every hour that passed after calving, colostral IgG concentration decreased by 3.7% (Morin et al., 2010). Moore *et al* demonstrated similar decreases in colostral IgG at milking over time (Moore et al., 2005).

Timing and volume of colostrum consumption: It has been well documented that Holstein dairy calves left to suckle their mother are at a much greater risk of FPT than those fed colostrum manually via a tube or teat (Besser et al., 1991; Brignole and Stott, 1980). This is due to a failure to consume a sufficient volume of colostrum within a sufficient time period if left with the dam. In support of this hypothesis Edwards demonstrated that 32% of Friesian calves failed to suckle within 6 hours of birth (Edwards, 1982). The timing of colostrum consumption is one of the most important factors governing passive transfer of Igs. Efficiency of transfer of Igs across the gut is maximal during the first 4 hours of life and declines progressively after 6 hours to closure of the gut wall at about 24 hours (Michanek et al., 1989; Weaver et al., 2000).

Practical advice on how to feed sufficient colostrum to new born calves varies widely but it is generally agreed that calves should receive 3 - 4 litres of colostrum *via* a teat, bucket or stomach tube within 6 hours of birth (Edmonson et al., 1989; Godden et al., 2009b).

Work to assess the behavioural impact of feeding large volumes of colostrum during the first feed has been carried out. One study reported that 47% of 244 calves refused to suckle their first milk replacer meal after being force fed 4 litres of colostrum (Anderson, 2011). The calves were fed by 2 routes, either nipple bottles or oesophageal feeders and the volumes fed varied. The study argues that force feeding of colostrum to calves may be stressful and needs exploring further; however the study did not compare the morbidity or mortality rates of these calves against animals that were not force fed colostrum.

1.6 Feeding the pre-weaned dairy calf

Naturally, calves would suckle their mother for 9 - 13 months after birth and consume around 10 litres of milk in 8 feeds per day (Albright and Arave, 1997; Reinhardt, 2002). Dairy calves are reared on milk replacer or waste whole milk, traditionally at a rate of 10% of body weight daily which equates to 4 litres daily for a 40 kg calf (Thomas et al., 2001). Milk feeds are usually split into 2 discrete meals, although some farmers use a once a day milk feeding regime (van der Burgt and Hepple, 2013). Although once a day milk feeding of calves is permitted by law (The Welfare of Farmed Animals (England, 2000), they must be over 28 days of age and a second non-liquid feed must be offered that is appropriate to satisfy nutritional needs. Although calves can perform well on once a day milk feeding strategies (Gleeson et al., 2007), there may be significant negative welfare implications.

Milk or MR feeding of young calves to 10% of body weight daily is unlikely to support growth during the first few weeks of life when intake of concentrate feed and forage is negligible (Figure 1.1). Furthermore, calves that have a high birth weight are likely to be fed the same volumes of milk as those of smaller birth weight, therefore being fed at less than 10% of body weight and undergoing even greater undernourishment.

40 kg calf

Maintenance requirements (ME) = 8 MJ

Growth requirements (0.7 kg/day, ME) = 10 MJ

Total requirement = **18 MJ**

4 litres milk replacer = **9 MJ/day**

Figure 1.1: Calculation of ME requirements for a 40 kg Holstein calf at one week of age in a thermoneutral environment (20°C) (National Research Council, 2001).

Brown adipose tissue (BAT) constitutes approximately 5% of body weight in the newborn human (Carter and Schucany, 2008), while Holstein calves are born with approximately 2% BAT (Alexander et al., 1975). This adipose tissue is brown in colour due to its increased vascularity, and proportion of mitochondria compared to white adipose tissue. BAT is involved in non-shivering thermogenesis of the newborn but once it is depleted, energy for thermogenesis and growth must be acquired from dietary intake. For dairy calves fed restricted volumes of MR, this dietary provision is often not met. The calf will begin to use body protein (lean tissue) to liberate the energy required for thermogenesis; growth will severely decrease, cease or reverse until sufficient dietary energy is ingested to once more enable growth.

The provision of restricted milk or MR for dairy calves is still commonplace on U.K. dairy farms and has been justified by anecdotal or short term evidence (Anderson, 2011). Early intake of concentrate feed promotes rumen development (Anderson et al., 1987). Studies have shown that concentrate feed intake is greater in restricted milk fed calves compared to *ad libitum* milk fed calves during the first few weeks of life, although no differences in post-weaning intakes were reported (Andreia De Paula et al., 2008; Borderas et al., 2009; Jasper and Weary, 2002). Veal calves are often fed *ad libitum* milk replacer in order to ensure sufficient Average Daily Gain (ADG) in weight prior to finishing. In addition to this, they are often fed low levels of concentrate feed, thus reducing the opportunity for rumen development (Webb et al., 2012). Another argument against *ad libitum* milk feeding of calves is that it causes an increase in diarrhoea; however many studies provide evidence

against this (Appleby et al., 2001; Chua et al., 2002; Diaz et al., 2001; Jasper and Weary, 2002). The occurrence of calf diarrhoea is related primarily to pathogen load in the environment in which the calf is kept (Roy and Ternouth, 1972). It is recognised that faecal scores will often be higher in *ad libitum* milk fed calves due to the greater fluid intake, although not significantly so. Similarly, feeding milk replacer rather than whole milk also gives rise to higher faecal scores (Bartlett et al., 2006).

There have been many studies that have assessed the effects of increased milk or MR feeding to neonatal calves (Table 1.1). Access to *ad libitum* milk or MR through teats mimics natural feeding, which is a factor that is missing in bucket fed systems. In 2001, Appleby *et al* (Appleby et al., 2001) reported that Holstein dairy calves fed *ad libitum* milk from a teat gained weight at a rate of 0.85 kg/day *vs*. 0.36 kg/day in conventional restricted twice daily bucket feeding. In another study, comparing *ad libitum* milk feeding with conventional systems, calves gained 63% more weight prior to weaning in the *ad libitum* group compared to calves in the conventional group (Jasper and Weary, 2002).

Drackley *et al* (Drackley et al., 2007) showed that calves fed *ad libitum* milk replacer and weaned at 6 weeks of age gained a weight advantage over calves fed restricted amounts. This advantage in weight had however disappeared by 12 weeks of age. The study went on to determine the amount of milk produced by all these animals during their first lactation and it was found that the *ad libitum* fed calves produced significantly larger volumes than that of the control animals.

There has been some evidence to suggest that calves fed increased energy and protein prior to weaning store more fat than calves fed a standard protein and energy diet (Brown et al., 2005b). However there is no evidence to suggest that this represents a risk in later life.

Studies have been carried out to assess the impact of type of housing, grouping of animals and amount of milk and concentrate feeding on the behaviour of pre-weaned calves. In one study, calves fed restricted volumes of MR rather than *ad libitum* during the pre-weaning phase of life spent less time lying down, more time at the feeder and visited the feeder more frequently than *ad libitum* fed calves (Andreia De Paula et al., 2008). This suggests that restricted fed calves are not as nutritionally satisfied as *ad libitum* fed animals. Other behavioural studies have highlighted the benefits associated with group rather than

individual housing (De Paula Vieira et al., 2010; Fujiwara et al., 2014). Concentrate consumption during early life is also increased in calves that are group housed rather than individually housed (Borderas et al., 2009; Hepola, 2003).

Table 1.1: Studies comparing *ad libitum* with restricted milk feeding in dairy calves.

Author	Year	*n/study*	Intervention	Outcomes
Appleby *et al.* (Appleby et al., 2001)	2001	23	*ad libitum* whole milk (teat) *vs*. restricted (10 % BW/day) from birth to 4 weeks	0-28 days: *ad libitum* fed calves consumed 87% more milk. 0-14 days: *ad lib.* 2.4 x weight gain14-28 days *ad lib.* 1.4 x weight gain
Borderas *et al.* (Borderas et al., 2009)	2009	1) 25 2) 28	Experiment 1: *ad libitum* (max 6L/feed) vs 4L MR/day Experiment 2: 4L vs. 6L whole milk daily	Experiment 1: ADG higher for *ad lib.* Fed calves from birth to 48 days Experiment 2: 6L fed calves had higher weight gain during first 28 days but no difference from 28 to 42 days
Drackley *et al.* (Drackley et al., 2007)	2007	10	MR at 1.25% BW/day till week 4, weaned at week 5 vs. 2-2.5% BW/day till week 5, weaned at week 6	Higher ADG for increased MR fed calves to week 4 (1.56 vs 0.66 lb/day) and 8 (1.52 vs 1.23 lb/day). However, no difference in week 12 body weight between dietary groups due to decreased growth at weaning in increased MR fed calves.
Jasper and Weary (Jasper and Weary, 2002)	2002	28	*ad libitum* vs. restricted (10 % BW/d) MR	Pre-weaning (0 - 35 d): 89% higher milk intake, 63% higher weight gain (10.5kg advantage). Post-weaning (d 63): 89.07±2.47kg vs 81.07±2.47kg.
Bar Peled *et al.* (Bar-Peled et al., 1997)	1997	40	Suckle dam every 8 hrs for 15mins/time vs 2L MR 2x/day	Age at conception: 394±15days vs 426±13days Conception rate: 83.4±10.4% vs 74.2±8.9%
Moallem *et al* (Moallem et al., 2010)	2010	46	Whole milk vs MR (0 - 60days). Free access 30mins 2x/day	Whole milk fed animals, age at 1st insemination, 23 days earlier. 1st lactation milk production 10.3% higher for Whole milk fed calves.
Richard *et al.* (Richard et al., 1988)	1998	42	*ad libitum* vs. 2 x daily cold acidified MR	Wk 1-5: *ad lib.* Consumed more MR than 2 x daily but no difference between groups for ADG
De Paula Vieira (Andreia De Paula et al., 2008)	2008	24	*ad libitum* vs. 10% BW daily of whole milk	8-14 days *ad libitum* gained 4 x as much weight as restricted and consumed twice as much milk.
Huuskonen (Huuskonen and Khalili, 2008)	2008	40	*ad libitum* vs. 6L MR daily	Pre-weaning *ad libitum* gained 690g/day compared to 543g/day in restricted group. During weaning, restricted gained more weight/day (1038g) than *ad libitum* fed calves (482g) No differences post-weaning
Kiezebrink *et al.* (Kiezebrink et al., 2015)	2015	152	4L whole milk *vs.* 8L whole milk per day	Calves fed 8L milk were heavier at 56 days with a greater ADG than calves fed 4L/day. No difference in 1st lactation milk yield

1.7 Housing

The optimal environment for a calf to be reared in is one that is draught free with sufficient ventilation to ensure humidity ranges of between 50 and 70% (Hill et al., 2011). The ability to efficiently thermoregulate during early life is compromised, so it is important that the calf remains in an environment that does not represent a thermal challenge. The thermoneutral zone for a new born calf is between 10 and 26°C and for a 1 month old calf is 0 to 23°C (Stull and Reynolds, 2008; Wathes et al., 1983). At temperatures outside this zone, animals may suffer from thermal stress. It is important to provide sufficient fresh bedding for animals to nest into and have access to fresh water at all times.

There are many types of calf housing; individual pens with or without solid sides, group pens with or without solid sides, individually or grouped in hutches with access to some outside space, or in a field. The most common methods used are individual pen or hutch housing and small group housing, each has its advantages and disadvantages (Table 1.2).

Table 1.2: Advantages and disadvantages of housing systems for dairy calves

Type of Housing	**Advantages**	**Disadvantages**
Individual pens- without solid sides	• Ability to monitor individual feed and water intake • Containment of disease (depending on proximity to other pens)	• Calves not able to move around freely or exhibit play behaviour
Individual pens- with solid sides	• Containment of disease • Ability to monitor individual feed and water intake	• Calves not able to interact with other calves or exhibit play behaviour
Group pens	• Calves able to move around freely • Calves able to play and interact with one another • Able to use an automatic group feeding system • Reduced labour time	• Containment of disease is not possible between individuals within the group • Automatic feeding systems may provide method of disease transmission between calves in the group
Individual Hutches	• Ability to monitor individual feed and water intake • Some room to move around • Calves can choose whether to be inside or outside	• Calves not able to interact and play • If hutches are outside, temperatures may fluctuate widely
Group Hutches	• Some room to move around • Reduces labour time • Calves can choose whether to be inside or outside • Calves can huddle together for warmth in cold weather	• Not able to contain disease between individuals within a group • If igloos are outside, automatic feeding systems may not be possible • Temperatures may fluctuate widely

In a study carried out by Chua *et al* (Chua et al., 2002), where all calves were fed *ad libitum* milk and gradually weaned at 5 weeks of age, calves individually housed suffered a growth check during the weaning period, whereas calves housed in pairs did not. Both groups of calves remained healthy throughout the study with no problems associated with *ad libitum* feeding.

Group housing of calves can have financial and welfare benefits (Fujiwara et al., 2014). Increased space for animals to move into warmer, more sheltered areas is especially beneficial in cold winter months, allowing calves to utilise energy for growth rather than for thermoregulation. In addition, they are able to socialise and become involved in play and physical activity, allowing for more natural behaviours to be expressed (Duve et al., 2012; Jensen and Kyhn, 2000). Disadvantages of group housing includes bullying of younger calves by their older counterparts (Hepola, 2003) and transmission of disease (Svensson et al., 2003), especially during the first few weeks of life when the risk of neonatal diarrhoea is high. Large group size is also associated with increased disease risk, current recommendations are to limit groups to a maximum of 6 - 9 calves (Svensson and Liberg, 2006). These factors play a significant role if there are large age ranges between calves in a group. Older calves cause a pathogen multiplier effect, infecting younger calves with less mature immune systems (Smith, 2003). If animals are grouped so that there is a range of no more than 2 weeks in one group, the above risks are minimised (Moser and Thomas, 2014).

1.8 Calf Behaviour

The impact of feeding system on the behaviour of dairy calves has been studied by various groups (Andreia De Paula et al., 2008; Appleby et al., 2001; Carla et al., 2010; De Paula Vieira et al., 2010; Fujiwara et al., 2014). De Paula Vieira *et al* (Andreia De Paula et al., 2008) reported that calves fed milk restricted to 10% of body weight daily were more active, more competitive and spent more time at the feeder than calves fed *ad libitum* milk. Increased vocalisation and activity of calves during the weaning period has been reported (Thomas et al., 2001). It is well known that the weaning period is a particularly stressful time for these youngsters (Weary et al., 2008). Animals that are grouped prior to weaning show better

growth rates than their individually housed counterparts (Bach et al., 2010). Along with behavioural changes during weaning, there are physiological changes that may occur. These include decreased hard food intake, a growth check and gastro-intestinal dysfunction (Jasper et al., 2008).

The gradual weaning of calves from milk onto solid food either by increasing the dilution of milk with water or by feeding smaller quantities can decrease the stress response of the calf during weaning (Jasper et al., 2008).

1.9 Weaning

Management decisions to determine onset of weaning for dairy calves may be based on a number of factors. Age, concentrate consumption, body size, convenience or a combination of these factors may be used. The DEFRA recommendation for Holstein calves is when starter intake is between 700 and 1000g per day for at least 3 consecutive days (usually 5-6 weeks of age). Weaning is very stressful for calves; this stress can be heightened if insufficient starter is being consumed (Sweeney et al., 2010).

Calves may be weaned from milk or MR using a variety of methods: abrupt, gradual decreases in volume or a gradual decrease in number of meals. Irrespective of the method used, weaning can be stressful for calves; growth rates and immune efficiency may be compromised for up to 3 weeks after weaning (Weary et al., 2008). Minimising stress by implementing gradual weaning strategies can help to reduce growth checks and disease risk during this period (Griebel and Hodgson, 2009; Jasper et al., 2008; Sweeney et al., 2010). Khan *et al* (Khan et al., 2007) reported weaning using a gradual 'step-down' method after allowing calves large volumes of milk in early life. This enabled a large increase in concentrate feed consumption during the weaning period and minimised the reduction in energy intake during this time. Furthermore, they found that calves fed a higher volume of milk had a heavier fore-stomach than calves fed restricted volumes of milk.

1.10 The impact of rearing strategy on: Rumen development

Calves are monogastric at birth and use only the abomasum and intestinal tract for digestion of consumed liquid feed until development of the rumen takes place (Laarman et al., 2012). The rumen of the new born calf is by-passed by closure of the oesophageal groove, stimulated during suckling of milk (Blowey, 1999). As the calf reaches 3 to 4 months of age, the relative size of the abomasum decreases from around 60% of whole stomach capacity to 20% and reduces further to around 8% in adulthood (Figure 1.2). This change is associated with increasing rumen development and function, so that in mature animals, the rumen is the primary digestive organ. Prior to weaning, the rumen of the calf must develop, become functional, and be able to absorb and metabolise volatile fatty acids (VFAs), the products of fermentation.

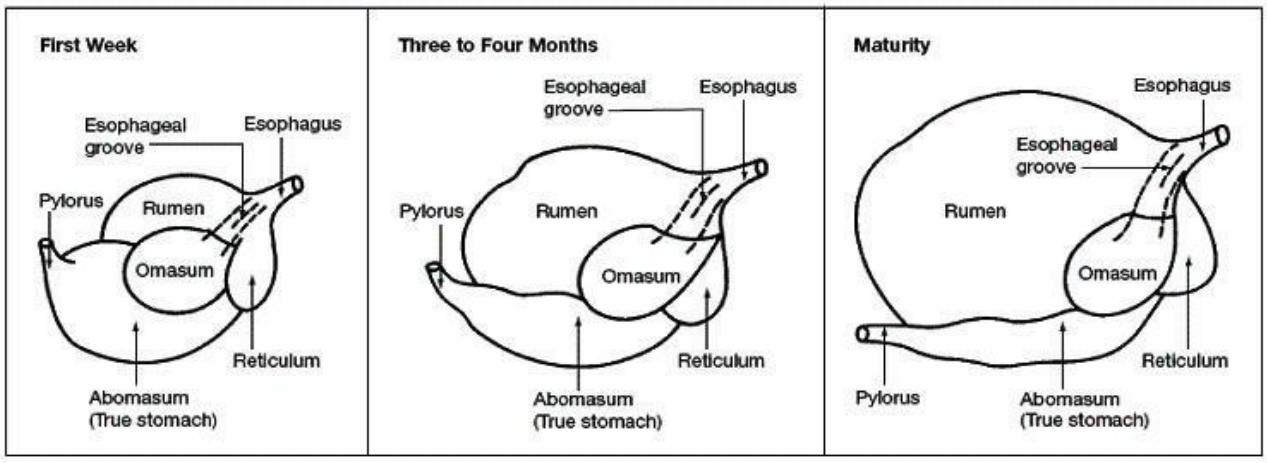

Figure 1.2: Diagrammatic representation of the bovine stomach compartments from birth to maturity (Picture taken from Eclipse Feeds Ltd website).

During the first week or so of life, a calf's rumen contains largely aerobic bacteria (Quigley, 2001). As the calf starts to consume solid food the bacterial population within the rumen starts to alter, shifting from an aerobic to an anaerobic population. The microbes that flourish in the rumen depend on the ingested substrate (whether it is predominantly forage or concentrate feed) (Coverdale et al., 2004; Lima et al., 2015). The presence of water within the rumen is crucial for bacterial growth, without which a sufficient bacterial population would not be grown to aid in the fermentation of substrates. Within 2 weeks of the start of

consumption of sufficient concentrate feed, the rumen microflora is similar to that of an adult cow (Bryant et al., 1958).

The primary VFAs produced from the fermentation process are acetate, propionate and butyrate; these are absorbed into the rumen epithelium. Propionate and butyrate aid in the development of papillae within the epithelial layer, increase the surface area of the rumen and enable further absorption (Harrison et al., 1960). If concentrate feed is not offered to calves during the pre-weaning period, there may be insufficient availability of energy from a solid diet immediately post-weaning due to poor rumen development. Rumen development should occur during the first 4 to 8 weeks of life.

In addition to concentrate feed, forage must be consumed by calves. Although intake of concentrate feed is important for the promotion of epithelial development, there is equal importance of increasing rumen volume and muscle development which is achieved by the presence of forage in the diet of the developing ruminant (Žitnan et al., 1998). A well-grown large muscular rumen is essential for maximising dry matter intake in the adult animal.
The lack of papillae development in milk only fed calves has been used as a justification for the use of restricted milk and early weaning strategies in dairy calves (Heinrichs and Lesmeister, 2005). However, the long term impact of this on productivity, health and longevity has not been assessed fully.

1.11 The impact of rearing strategy on: Mammary development

Optimal development of mammary parenchyma tissue is crucial for potential high yielding dairy cattle, without which, the genetic capability of milk production will never be realised and economic losses to the dairy producer may occur (Daniels, 2010).
The mammary gland of the dairy cow is made up of parenchymal cells (PAR) and a Mammary Fat Pad (MFP) (Sejrsen, 1994). At birth, PAR cells are present in negligible quantities and the MFP is very small, the teats are small and very close to the body wall. During the pre-pubertal phase of life, allometric growth of PAR occurs and they extend into the MFP. This rapid growth returns to an isometric rate after puberty (Daniels, 2010).

It has been reported that rapid growth rates in heifer calves, due to increased nutrition in early life, can cause a reduction in mammary development and an increase in fat deposition. Lammers *et al* (Lammers et al., 1999) found that Holstein heifers with an average daily gain of 1kg from 19 to 39 weeks of age had lower milk yields than heifers gaining 700g per day over the same time period. However this study compared these animals at the time of breeding. The animals that grew at a greater rate were younger than the slower growing heifers. It was therefore likely to be age associated differences in fat deposition rather than growth rate associated differences. In contrast, many studies have found that there is an increase of between 32 and 47% in mammary DNA content of calves fed twice as much milk replacer throughout the pre-weaning phase (1kg vs. 0.5kg per day) (Brown et al., 2005a; Meyer et al., 2006; Sejrsen, 1994). Sejrsen *et al* (Sejrsen, 1994) found no negative effect on mammary development when allowing pre-weaned calves access to *ad libitum* milk replacer.

There is clearly a lot of contradictory information available from various studies carried out. There have been suggestions that due to their proximity, there may be local cross-talk between PAR and MFP (Daniels, 2010). Further work is required in order to fully understand the biology of mammary growth and development and its implications in dairy heifers.

1.12 The impact of rearing strategy on: Disease and metabolism

The majority of dairy calf disease is associated with diarrhoea and pneumonia (Ballou and Eastridge, 2014; Virtala et al., 1996; Waltner-Toews et al., 1986; Windeyer et al., 2014). For diarrhoea, risk of morbidity and mortality is greatest during the first 3 weeks of life (Wells et al., 1996), and has been found to be closely related to management practices (Roy and Ternouth, 1972; Weary and von Keyserlingk, 2008) . As previously discussed, correct colostrum feeding is the basis for prevention of disease in the neonatal calf. However this must be followed with careful husbandry techniques to ensure optimal growth and minimal disease throughout life. Good colostrum management alone will not prevent infection from subsequent pathogen challenge.

The highest risk of pneumonia occurs at around 10 weeks of age (Lorenz et al., 2011). Adequate ventilation within housing areas is extremely important in the prevention of pneumonia due to excess humidity (Lago et al., 2006).

Many studies have highlighted associations between neonatal disease and management practices. One study, carried out during winter in a naturally ventilated barn, found a decreased incidence of neonatal respiratory in calves that were kept in pens with solid sides (Lago et al., 2006). They also found that calves with increased space to move around and nestle into their bedding had a lower prevalence of respiratory disease. Utilising solid sided partitions for pens removes direct contact between calves, creates individual pen "micro-environments" and minimises disease transmission, even though animals are sharing the same air space.

Type of feeding system may also influence disease transmission. One study found that calves housed in large groups and fed *via* an automatic feeding system had a higher odds ratio for respiratory disease (Svensson et al., 2003). The sharing of a teat from an automatic feeder can be an important mechanism of disease transmission and very careful management is necessary to ensure this rearing system is successful.

Incidence of neonatal disease in dairy calves has not changed over the past 20 years (Gorden and Plummer, 2010). In 2014 a study found that from a population of 2,874 heifer calves in Canada and the USA, 23 and 22% of calves were treated for at least one incidence of diarrhoea or pneumonia respectively (Windeyer et al., 2014). There was an overall mortality of 3.5% within the study population. Factors associated with increased risk of diarrhoea were weight of calves at enrolment (1-7 days of age), other diseases before 2 weeks of age, and an interaction between season of birth and herd level incidence of neonatal diarrhoea. Factors associated with the increased risk of respiratory disease were season of birth, whether the navel had been dipped, other disease prior to 2 weeks of age, FPT, and manual control of temperature in the calf house.

These studies highlight the importance of correct management, optimal housing and excellent hygiene in disease reduction and prevention in young calves. Poor building design leading to poor ventilation, increased humidity and overcrowding of accommodation can all increase the risk of neonatal disease (Lorenz et al., 2011). With the knowledge that has been acquired over years of research, it is disappointing that recent data has shown no reductions in disease rates on dairy farms.

1.13 The impact of rearing strategy on: Epigenetic factors

The term 'epigenetics' defines mitotic and meiotic changes in gene expression that are not coded in the DNA sequence itself (Dupont et al., 2009). These changes can be brought about by environmental factors but can also include genetic origins of behaviour. There are 3 systems that are involved in initiating and sustaining epigenetic silencing and expression; DNA methylation, RNA-associated silencing, and histone modification (Funston and Summers, 2013). The disruption of one or more of these systems (which interact) can give rise to 'epigenetic disease' due to inappropriate expression or silencing of genes. Epidemiological studies have been carried out in humans that were exposed to the Dutch Winter Famine of 1944. The offspring of famine exposed individuals were found to be at a higher risk of adult disease. It has been suggested that this is related to epigenetic dysregulation due to the decreased DNA Methylation of the IGF-2 gene in these subjects. This was compared to their same sex siblings (Heijmans et al., 2008) 6 decades later. This increased risk of disease was only found in individuals who were exposed to the famine during gestation.

There is evidence that people who are born with low birth weights or who have stunted growth during infancy and early childhood, but who show catch up growth later on, are at a much greater risk of becoming obese in adulthood and are also more susceptible to impaired glucose tolerance, diabetes, hypertension and cardiovascular diseases (Barker, 1998; Dulloo et al., 2002; Ozanne and Jones, 2009).

Similar findings have been presented from animal studies whereby suboptimal nutrition during the prenatal or neonatal phase of life may cause a similar state as seen in humans (Waterland, 2009). In terms of dairy cattle, the impact of early life events on the capacity for feed efficiency and future milk production could have large economic consequences. The evidence base for this is beginning to become apparent, with findings emerging in this area of study (Van Amburgh et al., 2011).

One study evaluated the association between milk yield of dairy cows over an 8 year period and early life growth and nutrition (Soberon et al., 2011). This study showed that for every 1kg of average daily gain (ADG), heifers produced 1067kg more milk during the first lactation. The authors concluded that it is possible to manipulate early life programming of these animals *via* increased nutrition. However, this manipulation must begin at birth and continue for at least the first 5 weeks of life. The authors also stated that in order to achieve

a positive influence on lifetime performance, this increased nutrition must be in the form of liquid feed (milk or MR).

1.14 The impact of rearing strategy on: Post-weaning growth

At weaning, it is imperative that the rumen is fully functional. This is necessary in order for the calf to be able to gain sufficient energy from solid foods to support growth. Many farmers aim to achieve growth rates of around 0.7 kg per day and it is important that growth checks immediately post-weaning do not occur.

Little emphasis has been placed on the importance of the period immediately after weaning in terms of nutrition for dairy heifers. They are often turned out into fields or fed 'waste' TMR from milking cow rations, resulting in sub-optimal feeding rations and encouraging the deposition of excess fat in these animals prior to first service. This may impact negatively on future health and productivity particularly if heifers are over-conditioned at calving (Bisinotto et al., 2011; Sinclair, 2010).
In contrast, improved growth during the rearing phase has been shown to be beneficial in terms of both future production (discussed earlier) and with respect to time of attainment of sexual maturity.

In a study carried out where groups of heifers were fed at either a standard (700g/day weight gain) or accelerated (1000g/day weight gain) feeding regime, animals fed the higher plane of nutrition achieved puberty more than a month earlier than the other animals (Lammers et al., 1999). This study was carried out on dairy heifers from 4.5 months of age over a 20 week period (Lammers et al., 1999). In order to benefit from this earlier attainment of sexual maturity, heifers should be served as soon as they are of an appropriate weight and size rather than waiting until animals are a certain age. There is debate on the optimal size and height at which heifers should be served, but recommendations of 55- 65% of mature bodyweight, approximately 380 - 400kg are common (Margerison et al., 2005). The danger of not serving heifers at target weight is that if service is delayed they may become over-conditioned, resulting in decreased fertility which will totally confound the positive benefits of enhancing growth rates during early life.

High morbidity and mortality rates of up to 40% in first calved heifers has been reported (Esslemont and Kossaibati, 1997), which is of huge financial loss to the dairy industry. Poor management of these animals as pre weaned and post weaned calves is a contributor to this loss, with both under and over conditioned animals being at increased risk. The age that a heifer calves for the first time is hugely important. The rearing of replacement heifers from birth amounts to approximately 20% of total farm costs (Dairy, 2011; Gabler et al., 2000). Published data recommend that the optimal age at first calving (AFC) is 24 months (Ettema and Santos, 2004; Haworth et al., 2008; Keown and Everett, 1986). It is therefore necessary to ensure that these animals enter their productive life as close to this time as possible. Below this age, heifers are unlikely to have sufficient body size to support their genetic potential for lifetime milk production or to easily deliver a healthy calf (Ettema and Santos, 2004). Conversely, rearing costs will be increased for animals with a greater AFC (Brickell et al., 2009a). Puberty of the Holstein heifer is attained at between 9 and 11 months. Some work has suggested that the age at first breeding can be as early as 11 months of age with an AFC of around 21 months. Provided heifers had reached the correct height and weight no adverse effects on ease of calving, reproductive efficiency or milk production were recorded (Corbett, 2010; Van Amburgh et al., 1998).

1.15 The impact of rearing strategy on: Survival of dairy heifers

Historically, farmers, nutritionists, vets and advisors have focused on milking cows and adult animals when optimising the profitability of a dairy herd. Recently, the focus has started to shift more towards calves and growing heifers as the future of the dairy herd. Measures of body weight, respiratory disease incidence, navel infections, and other markers such as conception rates can be used in young animals to predict the survival of a given animal (Bach, 2011). In one study, heifers reaching their second lactation had grown more between the ages of 12 and 65 days, had a lower average age at first calving and had a reduced incidence of respiratory disease (Bach, 2011).

The evidence regarding the impact of rearing strategies on future milk production is conflicting, In one study, heifers were fed one of 3 diets (Van Amburgh et al., 1998). The animals who grew most quickly, calved for the first time at 21.3 months compared to 24.5

months in the less well grown groups. However, the earlier calving heifers produced 5% less fat corrected milk in their first lactation (although this difference was not statistically significant). The authors did not report the performance of these animals in subsequent lactations. Other studies have reported reductions of up to 25% during first lactation after pre-pubertal growth rates of over 0.8kg per day. In contrast to this, other work has shown an increase in first lactation milk yield of up to 1300kg after *ad libitum* milk replacer feeding from birth until 56 days of life (Drackley et al., 2007; Raeth-Knight et al., 2008; Soberon et al., 2012).

1.16. Aims and Objectives of the Study

Although genetically capable of producing up to 32,000 litres of milk per year, the Holstein dairy cow rarely achieves this in practice. This is attributed to many factors, many of which are affected by early life events. In order to increase lifetime health and productivity through improved rearing of calves and youngstock, a solid evidence base is needed for best practice.

This study concentrated intensively on the neonatal nutrition and growth of the Holstein dairy calf and further assessed the health, growth and performance of these animals as they reach puberty, service and pregnancy for the first time. Increased feeding of milk replacer undoubtedly increases immediate growth rates of young calves, however the impact of this on future health and growth was assessed using key performance indicators. Using a cohort of 100 Holstein heifers born on one farm who will be retained on site for the whole of their productive life enabled un-interrupted collection of data throughout this study and beyond. Data gathered, and conclusions drawn from findings in this study will be utilised to ensure proven optimal animal husbandry and rearing strategies are offered to more of the industry's dairy animals.

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Chapter 2

Cross-sectional study to identify current dairy calf rearing strategies on U.K. dairy farms

2.1 Introduction

Sub-optimal dairy calf rearing has been commonplace for many years. This has been identified by high mortality rates of pre-weaned animals and has a huge economic impact (McGuirk, 2007). Data would suggest that although the issue is well recognised (Ortiz-Pelaez et al., 2008; Uetake, 2013; Wells et al., 1996), mortality rates have changed little over the last 40 years. In 1978, calf mortality was estimated at between 3 and 30% depending on geographical location (Greene, 1978). Today mortality rates are similar, with a range of between 1 and 20% in the U.K. at a calculated cost of around £60 million per annum (DEFRA, 2003).

Whilst historically, management of the dairy calf has been an area of little interest to farmers, the importance of correct husbandry is becoming more widely recognised and understood throughout the world. This has been illustrated *via* the vast amount of research being undertaken in this subject area in recent years (Brickell et al., 2009; Chang'a et al., 2010; DEFRA, 2003; Drackley, 2008; McGuirk, 2007; Stull and Reynolds, 2008; Wells et al., 1996).

Within the dairy industry, over half of neonatal deaths are related to diarrhoea (McGuirk, 2007). This is often a symptom of infection by pathogens such *as E. coli*, Rotavirus, Cryptosporidium and Salmonella and can be attributed largely to poor management and hygiene (Blowey, 1999).

Colostrum management is perhaps the most important technique to optimise in order to promote health in the dairy calf (Godden, 2008; Smith and Little, 1922; Weaver et al., 2000). Failure of the calf to consume and absorb sufficient concentrations of immunoglobulins (Ig) in the first few hours of life will leave them at great risk of disease during early life (Chigerwe et al., 2008; Godden, 2008; Weaver et al., 2000). The success of passive transfer of sufficient Ig will greatly “immunologically” aid the young calf when inevitably challenged with disease causing pathogens in the environment into which they are born (Furman-Fratczak et al., 2011).

In addition to colostrum administration factors such as adequacy of milk and concentrate feeding, housing, stocking density and hygiene have a large impact on the health status of

calves both prior to and after weaning (Godden, 2008; Vasseur et al., 2010; Wathes et al., 1983).

A female dairy calf reared for future milk production will not become profitable until mid-way through lactation two due to costs associated with rearing up to first calving (Wathes, 2012). With approximately 20% of total production costs attributed to heifer rearing, the lifetime productivity of a dairy animal is key to determining the profitability of a dairy enterprise (Bach, 2011a).

The largest U.K. supermarkets ensure sustainability and quality of milk products to their stores by the development of their own supply chain consisting of a select number of farmers. The Tesco Sustainable Dairy Group (TSDG) consists of approximately 700 farmers. The prices paid to these farmers are based on the cost of production, which has resulted in TSDG producers receiving higher prices for their milk compared to their non-Supermarket contracted counterparts.

It is the intention that farmers signed to supermarket contracts have the added security of extra income to allow expansion of their enterprises, provide optimal animal welfare conditions or to promote other areas of their business. In return for this increase in milk price, the Supermarkets have strict quality and welfare codes that farms must adhere to. The Tesco Code of Practice for dairy farmers was renewed in 2011, allowing farmers to benchmark themselves against other farmers within the TSDG and to record antimicrobial usage, lameness and mastitis events as well as other welfare parameters.

The high mortality rates (> 5%) (Brickell et al., 2009) observed on many U.K. dairy farms would suggest calf rearing practices are sub-optimal; this has a huge impact on future health and profitability of individual animals. The collection of up to date information regarding current rearing strategies employed by farmers throughout the U.K. will give a useful insight into how potential advances may be made to improve health and welfare of neonatal calves on farms.

The aims of this study were to determine the current dairy calf rearing strategies on farms in the U.K. and to investigate any associations between management practices of calves and performance of adult animals such as milk yield and cull rates.

A secondary objective was to test the hypothesis that there are differences in farm management practices between dairy farms contracted to supply milk to a retailer as a member of a supply group, in this case the TSDG, and farms which sell milk on the open market (non-TSDG).

2.2 Materials and Methods

Study Design

A postal questionnaire of calf management practices, breeding policies and adult cow performance was sent to a subset of U.K. dairy farms. The study population was dairy farms that supplied milk to the three largest U.K. liquid milk processors (Arla Ltd, Müller Wiseman Ltd and Dairy Crest Ltd). These companies process liquid milk for all farms within the TSDG as well as a larger number of non-TSDG farms. A total of 1000 questionnaires were delivered to randomly selected farmers. The questionnaire consisted of 66 questions that covered demographics, calf management practices, fertility, vaccination policies and animal housing at various ages (Appendix A), an overview of questions within the survey are shown in Table 2.1.

Table 2.1: An overview of information recorded in the postal questionnaire to dairy farmers.

Section of Questionnaire	**Questions asked**
Staff	Total number of farm staff Number, age, sex and experience of calf staff Weekend staff
Herd	Numbers of adults, young stock and calves Milk yield
Fertility	Calving pattern Calving index Cull rates Breeding policy Bull breeds used
Vaccination	Adults and calves, vaccines used
Management systems	High/Low input Housing of milking cows, dry cows, growing stock and in calf heifers
Calving management	Where animals calve down Type of bedding Cleaning and disinfection of calving area
Newborn calf	Time with dam Bull calves Colostrum management
Calf housing	Type of housing used Number of calves per group Age at grouping Bedding used and cleaning and disinfection
Calf feeding	Type of milk fed (replacer, waste milk etc.) Concentration and volumes fed Method of feeding (machine, bucket etc.) Storage of milk Cleaning of feeding equipment Concentrate and forage feed
Other calf management	Medication given to calves routinely Weighing and measuring of height, belly girth etc.) When calves are weaned-criteria used and method of weaning Post weaning grouping

Statistical Analysis

Raw data was initially entered into a database (Epidata 3.1 :EpiData Association, Denmark) and subsequently exported to STATA 13 (StataCorp, Texas, U.S.A.) for statistical analyses. Summary statistics were generated for all variables with nominal data categorised as required. Nominal response data was examined graphically for normality using the normal quantile plots. Summary statistics are presented as means or percentages with 95% confidence intervals (CI) where appropriate. Non parametric data is presented as medians with inter-quartile ranges. Chi squared tests and Student t tests were used to examine responses by type of farm (TSDG or non-TSDG). The impact of herd size was examined in a similar manner. Linear and logistic regression techniques were used where appropriate. Predicted marginal means were estimated from regression models and are presented graphically. Where results are presented to a specific question, the denominator is the number of farmers who answered the specific question not the number of farmers who returned questionnaires.

2.3 Results

Of the 1000 questionnaires sent out to farmers, there were a total of 723 responses (72%) with 207 responses from non-TSDG contracted farms and 516 from TSDG farms. On Arla farms, 205 out of 296 TSDG farms responded (69%), whereas only 91 out of 186 non-TSDG farms responded (49%) to the postal questionnaire. On Müller Wiseman farms 356 out of 460 farms responded (77.4%). Unfortunately, not all farmers responded to all of the questions asked within the questionnaire.

Farm Staff

The median number of workers on a farm was 3 (IQR 2 -4, range 1-48), with no differences between TSDG and non-TSDG groups. The majority of farms had 1 or 2 people looking after calves on a regular basis (643 farms, 89.4%) with 40% of farms indicating that it was the farm manager who looked after the calves regularly.

On both TSDG and non-TSDG farms, the majority of staff caring for the calves had over 10 years of experience (74.6 %, 95% CI 71.3 – 77.8). There were more staff with less than one year's experience employed on TSDG compared to non-TSDG farms (2.9% versus 0.5%, Figure 2.1). There were no differences between TSDG and non-TSDG farms in terms of the age of calf carers, with 230 farms (33%) having staff aged between 41 and 50 years. On 235 farms (33%), there were different calf carers at the weekends compared to the week days.

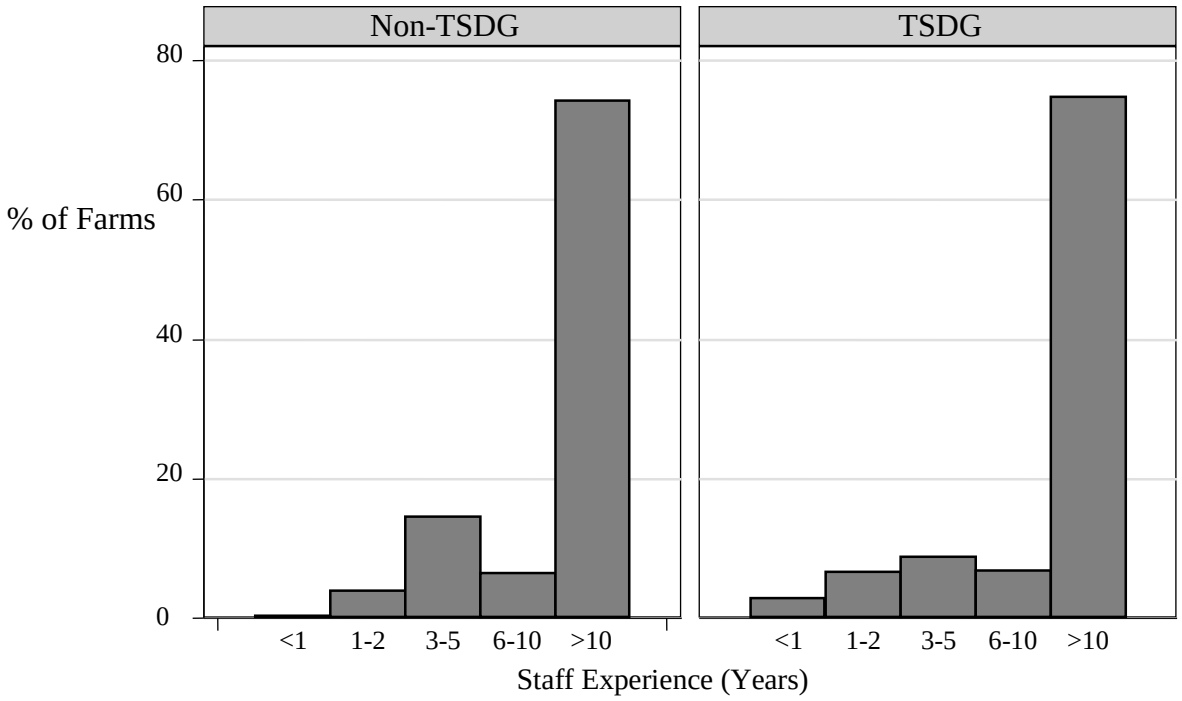

Figure 2.1: Number of years of calf caring experience by staff on TSDG and non-TSDG farms.

The Dairy Herd

The mean herd size (defined as adult lactating cows) was greater on TSDG farms (175.6 animals, 95% CI 165.6 – 185.5) compared to non-TSDG farms (156.7 animals, 95% CI 146.3 – 167.2, P = 0.015). This however, was not reflected in the number of young animals less than 2 years old, with no difference between TSDG and non-TSDG farms (128.0, 95% CI 120.1-135.9). After adjusting for herd size using regression techniques, there was a significant difference in youngstock numbers detected between farm types. Farms within the TSDG kept significantly less young stock than their non-TSDG counterparts (P = 0.012) (Table 2.2).

Table 2.2 shows that for a herd size of 100 cows, there will be (100*0.76 + 9.4) = 85 young stock on a non-TSDG farm compared to (100*0.76 + 9.4 – 14.2) = 71 youngstock on a TSDG farm.

There was no significant difference (P =0.499) between TSDG and non-TSDG farms in terms of numbers of animals purchased. The majority of farms (299, 42%) purchased between 1 and 20% of the animals (15.3%, 95% CI 13.3 – 17.3) within their herds. Overall, 279 (39%) farms claimed to be of closed herd status whilst 29 farms (4.1%) were "flying herds", whereby all lactating animals were purchased with no replacements being reared. .

There were no significant differences between TSDG and non-TSDG farms with regards to the breeds of animals kept: 645 (89%) of all farms kept Holstein-Friesians, 96 (13%) kept crossbred animals, 32 (4%) kept Channel Island breed animals, 179 (25%) kept British Friesians and 74 farms (10%) kept other breeds or a combination of the above.

Of the 723 respondents, 14 (1.9%) did not supply details of annual milk yield per cow. There was no significant difference in reported annual milk yield per cow between the two groups of farms (P = 0.828), with mean annual milk yield across all farms being 8244 litres (95% CI 8157 – 8331) per animal. However there was a positive association between milk yield and herd size (P < 0.001) (Figure 2.2).

Table 2.2: Association between the total numbers of youngstock kept on farms, adult population size and TSDG membership.

Total Youngstock	**Regression Coefficient**	**95% Confidence Interval**	**P value**
TSDG membership	-14.2	-25.2 - -3.2	0.012
Number of adult cows	0.76	0.72 – 0.81	< 0.001
Baseline	9.4	-2.3 – 21.1	0.12

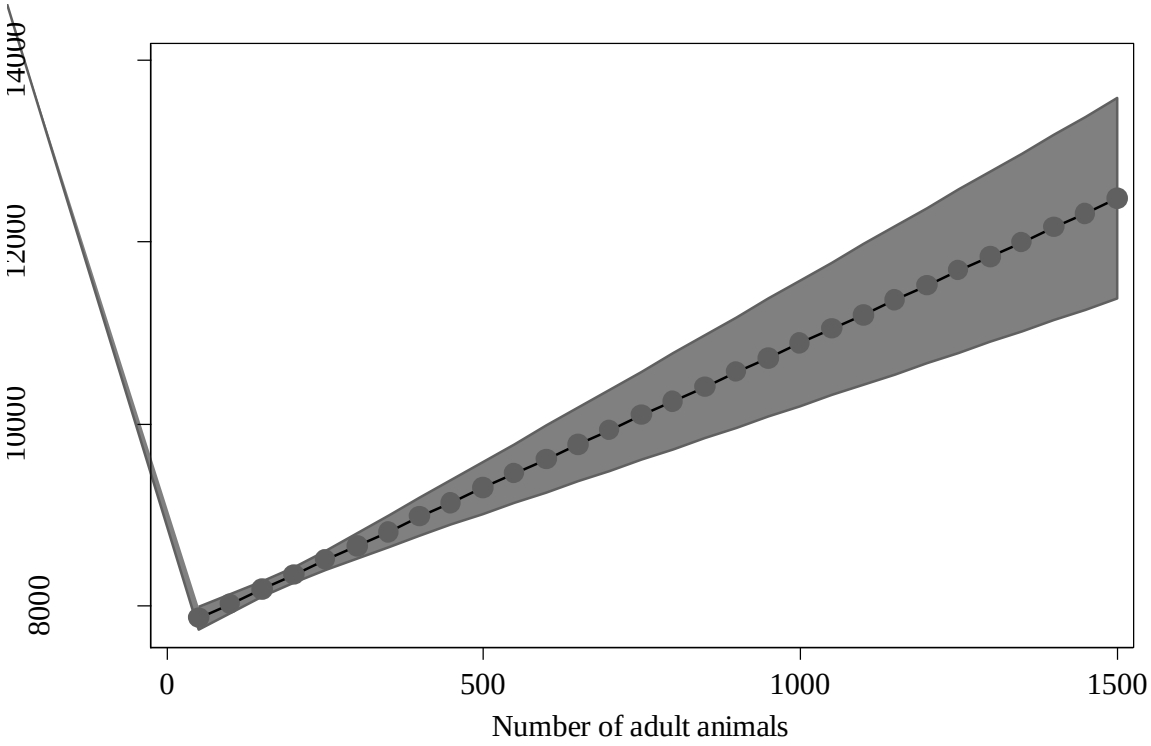

Figure 2.2: Predicted marginal means (95% CI) for annual milk yield per cow and herd size.

Fertility

The response rate pertaining to questions regarding fertility performance was less than 100%. Questions regarding calving index and cull rates of cows and heifers were answered by 91%, 92% and 88% of farmers respectively. Fifty-five percent of farms (365) had a calving index of between 401 and 450 days (Figure 2.3). The mean calving index on non-TSDG farms was 403 (95% CI 400 – 407) days compared to 409 days (95% CI 407 – 411) on TSDG farms (P = 0.002). Regression analysis demonstrated that herd size had no impact on calving index, although there was a positive association between calving index and increasing annual yield per cow (Figure 2.4).

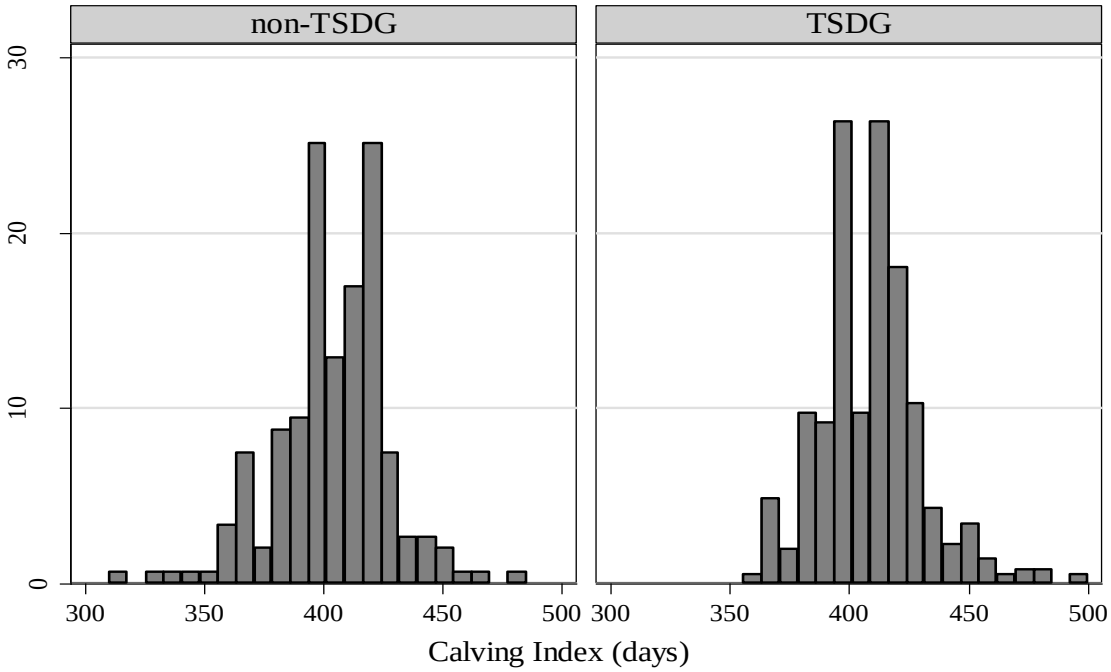

Figure 2.3: Comparison of Calving index for TSDG and non-TSDG farms.

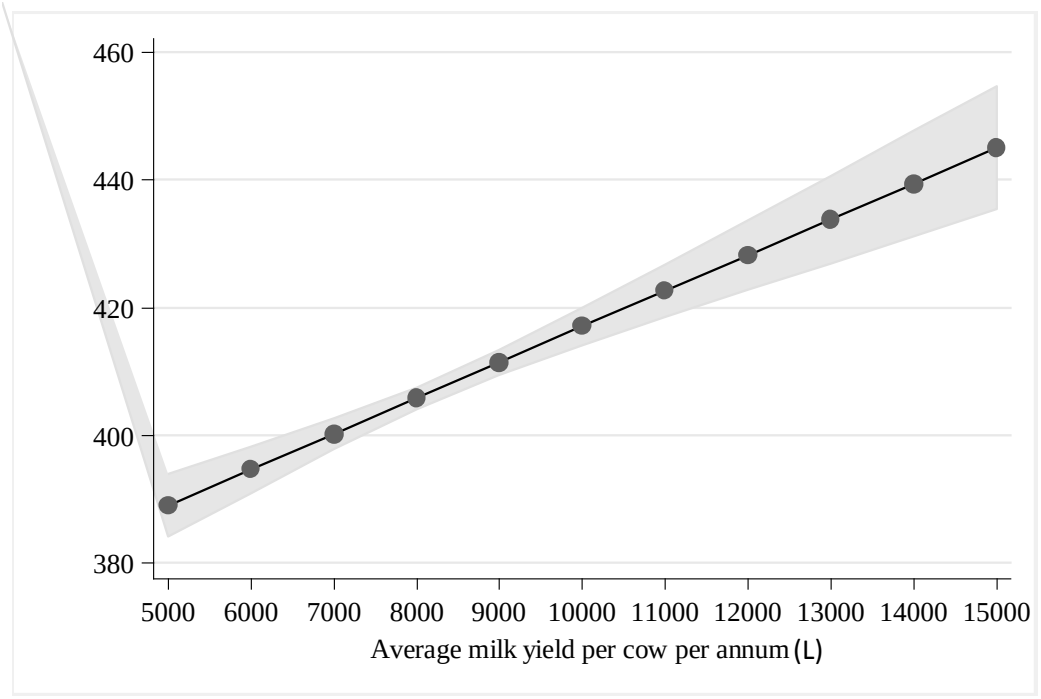

Figure 2.4: Predicted marginal means (95% CI) for annual milk yield per cow and calving index.

The mean cull rate for cows was 19.5% (95% CI 19.0 - 20.0) and was not significantly different between TSDG and non-TSDG farms (P = 0.095, Figure 2.5). The cull rate for first calving heifers was much lower than that of cows with 66% (419) of farms having cull rates of less than 5%. Only 9 farms (1.4%) had first calving heifer cull rates over 20%.

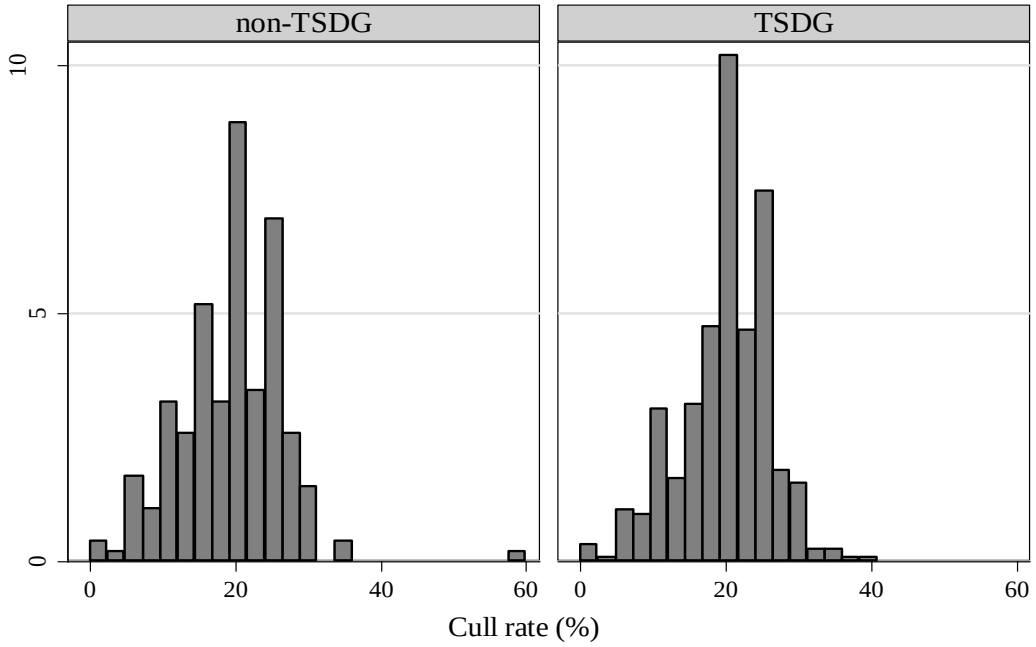

Figure 2.5: Adult cow cull rates on TSDG and non-TSDG farms.

Regression analyses (Table 2.3) suggested that there was a positive association between adult cull rate and both calving index and herd size. Figure 2.6 a & b shows that there was a positive association between both the number of adult animals and calving index with cull rate.

Table 2.3: Association between adult cull rate and both calving index and herd size.

Adult cull rate	**Regression Coefficient**	**95% Confidence Interval**	**P value**
Calving index	0.058	0.037 - 0.080	<0.001
Number of adults	0.018	0.013 - 0.022	<0.001
Baseline	-6.989	-15.763- 1.784	0.118

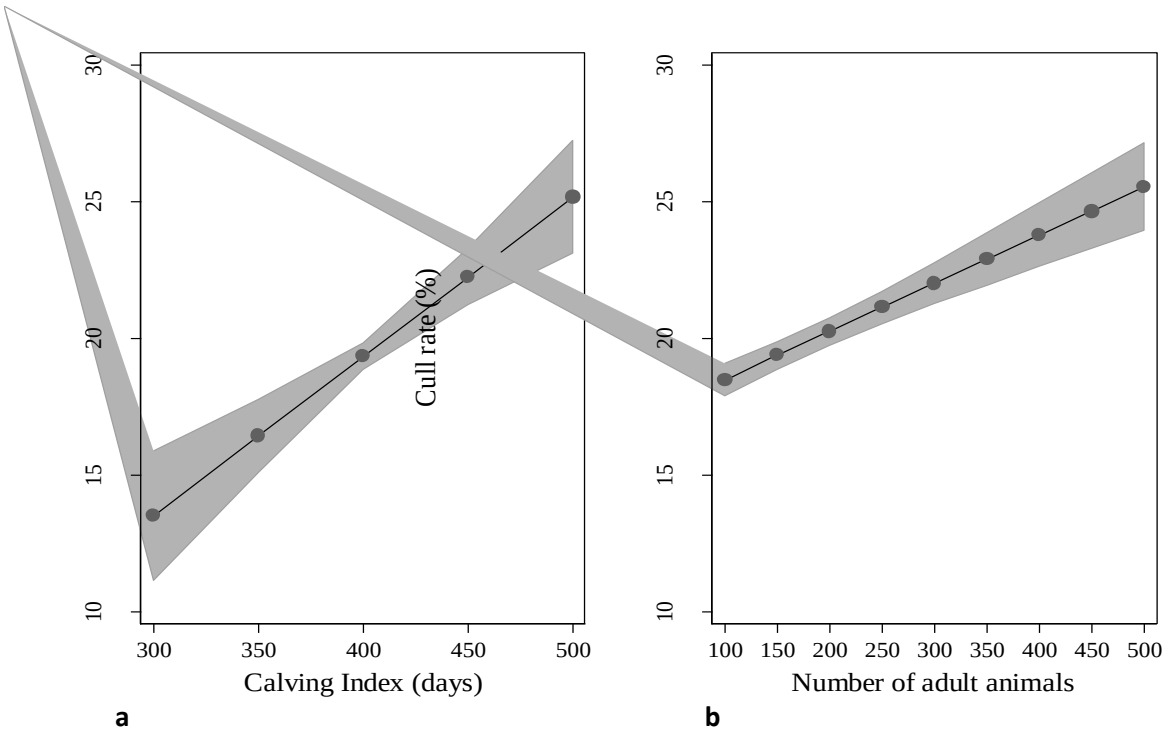

Figure 2.6a & b: Predicted marginal means (95% CI) for a) calving index and cull rate and b) number of adult animals and cull rate.

Whilst breeding policies (use of AI or bull service, or both) varied between individual farms, there were no differences between TSDG and non-TSDG farms. Fifty four (7.6%, 95% CI 5.6 - 9.5%) farms used bull service only, 203 (28.4%, 95% CI 25.1 - 31.7%) farms used artificial insemination alone, with the majority (457 (64%, 95% CI 60.5 - 67.5%)) using both service methods. Three-quarters of all farms (533, 95% CI 72.1 - 78.5%) used both dairy and beef bulls for breeding their adult cows, with no difference between the TSDG and non-TSDG farms (P = 0.121). In the case of heifers, 270 of all farms (40%, 95% CI 36.5 - 43.9) used dairy sires whilst 277 (41%, 95% CI 37.5 - 45.0) used both dairy and beef sires and the remainder (19%, 95% CI 15.5 - 22.8) used beef sires only.

Vaccination

Vaccination of adult cows was carried out on 602 of the 709 farms (85%) who answered this question, although details regarding vaccine type were not available for all farms. Where type of vaccine delivered was specified, there were significant differences apparent between TSDG and non-TSDG farms. Infectious Bovine Rhinotracheitis (IBR) vaccine usage was significantly higher for TSDG farms compared to non-TSDG farms, with 232 (45%, 95% CI 40.7 - 49.3) TSDG farms vaccinating compared to 66 (32%, 95% CI 25.5 - 38.3) non-TSDG farms (P = 0.001). However this was not the case for vaccination against Leptospirosis (P = 0.758) or Bovine Viral Diarrhoea (BVD) (P = 0.229). Four hundred and thirty six farms vaccinated against Leptospirosis (60.3%, 95% CI 56.7 – 63.9) and 468 farms vaccinated against BVD (64.7%, 95% CI 61.2 – 68.2). For all vaccine types there was a positive association between vaccination and milk yield per cow:

- IBR vaccine 8504 litres (95% CI 8375-8634) versus 8058 litres (95% CI 7944 - 8173) P < 0.001
- BVD vaccine 8443 litres (95% CI 8345-8542) versus 7870 litres (95% CI 7711 - 8030) P < 0.001
- Leptospirosis 8419 litres (95% CI 8319-8520) versus 7971 litres (95% CI 7817 - 8125) P < 0.001

Other vaccines reported as being administered to adult and growing cattle were: Salmonella (13 farms, 1.8%), Bluetongue (59 farms, 8.1%), Blackleg (3 farms, 0.4%) and Lungworm (10 farms, 1.4%).

Two hundred and fifty two respondents (37.1%, 95% CI 33.5 - 40.8) reported vaccinating calves, with no difference in uptake between TSDG and non-TSDG farms (P = 0.912). Only 97 (13.4%, 95% CI 10.9 - 15.9) farms vaccinated calves for Rotavirus, again with no difference in uptake between TSDG and non-TSDG farms (P = 0.591). One hundred and ninety three respondents (26.7%, 95% CI 23.5 – 30.0) reported vaccinating calves against pneumonia, with no difference in vaccination rate between TSDG and non-TSDG farms (P = 0.125). There was a positive association between calf vaccination and milk yield:

- Rotavirus vaccine 8503 litres (95% CI 8243 - 8761) versus 8203 litres (95% CI 8110 - 8295) P = 0.010
- Pneumonia vaccine 8491 litres (95% CI 8348 - 8633) versus 8121 litres (95% CI 8007 - 8235) P = 0.052

Management Systems

Respondents were asked to classify their enterprise by the ratio of input to output. The majority of respondents (*n* = 447 62.9%, 95% CI 59.3 - 66.4) classified their enterprises as medium input: medium output. The type of management system employed by farms showed significant differences between TSDG and non-TSDG farms (P = 0.026). Significantly more respondents who classified their enterprise as a low input: low output enterprise belonged to the non-TSDG group (*n* = 25 12.2%, 95% CI 7.7 - 16.7) than the TSDG group (*n* = 35 7%, 95% CI 4.7 - 9.1). A small number of farms (*n* = 29, 4.1% 95% CI 2.6 - 5.5) from both the TSDG and non-TSDG groups housed their milking cows all year round. As would be expected there were highly significant differences (P < 0.001) in annual milk yield per cow between the three systems (Table 2.4).

Table 2.4: Association between mean annual milk yield per cow (L) and type of farming system classified by farmers.

Type of farming system	**Number of farms**	**Mean annual milk yield per cow (L)**	**95% Confidence Interval**
High input: high output	204	9404	9290-9517
Medium input: medium output	447	7949	7873-8025
Low input: low output	60	6361	6144-6577

The majority of respondents (*n* = 616, 86.3%, 95% CI 83.7 - 88.8) reported housing cows in winter and turning them out to pasture in summer, irrespective of whether they were TSDG or non-TSDG farmers. For housing of animals at various stages of their lives, there were no differences between TSDG and non-TSDG farms.

Lactating cows were mostly housed in either cubicles alone (*n* = 390 54.4%, 95% CI 50.8 - 58.1) or in both cubicles and yards (*n* = 268 37.4%, 95% CI 33.9 - 41.0), with very few farms using straw yards solely for these animals (*n* = 58 8%, 95% CI 6.1 - 10.1). Dry cows were housed mainly in both cubicles and yards (*n* = 266 37.4%, 95% CI 33.8 - 40.9) or in straw yards solely (*n* = 252 35.4%, 95% CI 31.9 - 38.9). Cows in the transition period were similarly housed with 156 farms (24.2%, 95% CI 20.9 - 27.5) using cubicles and yards and 377 farms (58.4%, 95% CI 54.6 - 62.2) utilising straw yards respectively.

On 487 (67%, 95% CI 63.9 - 70.8) farms, youngstock had no access to pasture in the period up till first calving. TSDG farmers (*n* = 359 69.6%, 95% CI 65.6 - 73.6) were less likely (P = 0.450) to allow access to pasture than non-TSDG farms (*n* = 128 61.8%, 95% CI 55.2 - 68.4).

Table 2.5: Housing types used for heifers on TSDG and non-TSDG farms (*n* = number of farms, % = percentage of farms).

	Housing Type											
Animal Category	**Straw Yard**			**Cubicles and Yard**			**Cubicles only**			**Not Housed**		
	n	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Growing Heifers (<12mo.)	377	56.3	52.5 - 60.0	219	32.7	29.1 - 36.2	68	10.2	7.9 - 12.4	6	0.9	0.1 - 1.6
Bulling heifers (> 12 mo.)	275	41.1	37.4 - 44.8	142	21.2	18.1 - 24.3	242	36.2	32.5 - 39.8	10	1.5	0.6 - 2.4
In calf heifers (13-30mo.)	227	33.8	30.2- 37.4	181	27.0	23.6 - 30.3	254	37.9	34.2 - 41.5	9	1.3	0.4 - 2.2

Heifers from 12 months of age until calving were housed in straw yards or cubicles with many farmers employing both systems (Table 2.5). There was no significant difference between TSDG and non-TSDG farms with respect to methods of housing of heifers.

Calving Management

Overall there was considerable variation between individual farms in management and housing of the calving cow or heifer, but there were no significant ($P < 0.05$) differences between TSDG and non-TSDG farms.

During the summer months, 355 (49.7%, 95% CI 46.0 - 53.3) farms reported calving cows both inside and outside whilst 159 farms (22.2%, 95% CI 19.2 - 25.3) calved animals down only inside. There were a further 201 farms (28.1%, 95% CI 24.8 - 31.4) that calved cows solely outside during the summer months.

In contrast to this, during the winter all farms calved animals inside utilising a mixture of housing systems. Three hundred and twenty three farms (46.2%, 95% CI 42.5 - 49.9) reported calving animals in groups whilst 212 farms (30.3%, 95% CI 26.9 - 33.7) used individual calving boxes. One hundred and forty two farmers (20.3%, 95% CI 17.3 - 23.3) reported that they calved cows in the dry cow accommodation.

A total of 395 (54.6%, 95% CI 51 - 58) farmers reported having individual calving boxes on their farms with no differences detected between TSDG and non-TSDG farms ($P = 0.330$). Of the farms that had calving boxes, 245 (63%, 95% CI 58.3 - 68.0) reported having between 2 and 4 boxes and only 25 (6.4%, 95% CI 4.0-8.9) having over 8 boxes. The bedding material used for calving boxes was straw on most farms (*n* = 385 96.7%, 95% CI 95.0 - 98.5), and new bedding was added to the pen for each calving on 297 farms (76.9%, 95% CI 72.7 - 81.2). The calving boxes were completely cleaned out after each calving on 66 farms (16.9%, 95% CI 13.2 - 20.7) and otherwise monthly (*n* = 123, 31.5% 95% CI 26.9 - 36.2), between 3 to 6 calvings (*n* = 114, 29.2%, 95% CI 24.7 - 33.8), or annually (*n* = 3, 0.7%, 95% CI 0.1 - 1.6) on the remainder of farms. Just under half (*n* = 327, 45% 95% CI 41.6 - 48.9) of all farmers reported disinfecting calving boxes. When group housing or dry cow accommodation was used as a calving area, 446 farms (97.9%, 95% CI 96.5 - 99.2) used straw as bedding material,

with just over half of these farms (*n* = 244 56.4%, 95% CI51.7 - 61.0) using sterilising compounds. The group size in these areas utilised for calving ranged from 1 to over 31 cows, with 289 farms (63.4%, 95% CI 58.9 - 67.8) having between 2 and 10 cows per group and 119 farms (26.1%, 95% CI 22.1 - 30.1) having between 11 and 20 cows per group. Farmers added new bedding to group calving areas on a daily basis in 302 cases (65.8%, 95% CI 61.4 - 70.2), with 132 farms (28.8%, 95% CI 24.6 - 32.9) adding fresh straw every second day.

The Newborn Calf

Although there was considerable variation in aspects of management of the newborn calf, there were no significant differences (P< 0.05) between TSDG and non-TSDG farms in this area.

There was considerable variation in the length of time the newborn calf was left with its dam prior to removal to calf housing (Table 2.6).

Table 2.6: The length of time that newborn calves were left with the dam on both TSDG and non-TSDG farms.

Time calf left with dam (hours)	Number of farms	% of farms	95% Confidence interval
< 3	43	6.0	4.2 - 7.7
3 - 6	81	11.3	9.0 - 13.7
6 - 24	208	29.1	25.8 - 32.4
24 - 48	218	30.5	27.1 - 33.9
48 - 96	107	15.0	12.3 - 17.6
> 96	58	8.1	6.1 – 10.1

The navel of the newborn calf was dipped on 519 (71.8%, 95% CI 68.5 - 75.1) farms. Dairy bull calves were euthanased at birth on 10.4% of farms with a tendency for non-TSDG farms (*n* = 27 13.3%, 95% CI 8.6 - 18.0) to be more likely to adopt this policy than TSDG farmers (*n* = 47 9.2%, 95% CI 6.7 - 11.8; P = 0.090). Forty eight percent of farms sold bull calves between 7 and 21 days of age (*n* = 343 48%, 95% CI 44.5 - 51.9) whilst 11.2% (*n* = 80, 95% CI 8.9 - 13.6%) reared bull calves until weaning. Two hundred and fifteen farms (30.2%, 95% CI 26.8 - 33.6%) reported that they kept bull calves for longer than this.

There was considerable variation in how farmers delivered colostrum to newborn calves. Five hundred and forty nine farmers (75.2%, 95% CI 72.0 - 78.4) reported that they always allowed calves to suckle from their dams, with 138 farmers (19.2%, 95% CI 16.3 - 22.1) allowed suckling sometimes. Only 40 (5.6%, 95% CI 3.9 - 7.2) farmers reported that they did not allow calves to suckle the dam. Two hundred and ninety (40.8%, 95% CI 37.2 - 44.4) farmers reported feeding all calves with colostrum, whilst 373 (52.5%, 95% CI 48.8 - 56.1) farmers allowed natural suckling of the dam and only fed calves with extra colostrum if the farmer thought necessary. A small percentage (*n* = 48, 6.8%, 95% CI 4.9 - 8.6) did not manually feed colostrum at all and relied solely on sufficient suckling by the calf from its dam. Of the farmers who actively administered colostrum to calves, 272 (38.7%, 95% CI 35.1 - 42.3) used a stomach tube, whilst 365 (51.7%, 95% CI 48.0 - 55.4) used a bucket and teat or a bottle and teat. On the majority of farms (*n* = 621 88.0%, 95% CI 85.6 - 90.4), the colostrum fed to calves came exclusively from the calf's own dam, whilst on 52 (7.4%, 95% CI 5.4 - 9.3) farms, colostrum from other cows was often administered. A further 33 (4.7%, 95% CI 3.1 - 6.2) farms gave pooled colostrum from multiple cows.

The timing of colostrum consumption after birth varied between farms and depended, in part, on the time the calf was born (day versus night). Significantly less farmers feed colostrum within 3 hours of birth if the calf is born during the night (*n* = 59 8.9%, 95% CI 6.7 - 11.1) compared to daytime (*n* = 225 32.1%, 95% CI 28.6 - 35.6: P < 0.001). Regardless of the time of birth, colostrum was fed within 6 hours of birth on 392 (55.6%, 95% CI 51.8 - 59.4) of all farms. A smaller number of farmers fed colostrum at between 6 - 12 hours of birth, again with a marked difference depending on the time of day that the calf was born. Seventy six (10.8%, 95% CI 8.5 - 13.1) farmers fed colostrum during this period if calves were born during the day, compared to 230 (34.7%, 95% CI 31.1 - 38.4) farms feeding colostrum

between 6 and 12 hours of birth if the calf was born at night. Very few farms tested the quality of colostrum fed to calves (*n* = 74, 10.2%, 95% CI 8.0 - 12.4), whilst only 43 (5.9%, 95% CI 4.2 - 7.7) farms routinely tested calves for the level of passive transfer of immunoglobulins.

Calf Housing

Again, whilst there was considerable variation in calf housing practices between farms, there were no significant differences ($P < 0.05$) between TSDG and non-TSDG farms in for housing of calves.

The majority of farms in this study housed calves in designated calf houses (*n* = 594, 83.2%, 95% CI 80.4 - 85.9), however, there were a small proportion of farms that used buildings that were shared with adult animals (*n* = 73, 10.2%, 95% CI 8.0 - 12.5). There was a statistically non significant ($P = 0.170$) tendency for more TSDG farmers to use shared buildings (*n* = 59 11.6%, 95% CI 8.8 - 14.4) than non-TSDG farmers (*n* = 14 6.8% 95% CI 3.4 - 10.2). There was considerable variation in type of calf housing used for both pre-weaned and post-weaned calves, and the age at which calves were moved from individual to group housing.

Three hundred and twenty two farmers (44.5%, 95% CI 40.9 - 48.2) reported housing pre-weaned calves in individual pens whilst 457 (63.2%, 95% CI 59.7 - 66.7) housed these calves in groups. A further 85 (11.8%, 95% CI 9.4 - 14.1) farms housed pre-weaned calves in individual calf hutches. Amongst farmers who grouped their pre-weaned calves, there was considerable variation in age at grouping (Table 2.7).

Table 2.7: Age at which pre-weaned calves were introduced to grouped accommodation.

Age at grouping (days)	*n*	% of farms	95% Confidence intervals
0	39	7.6	5.3 – 9.9
1	35	6.8	4.6 – 9.0
2	53	10.3	7.6 – 13.0
3 – 7	182	35.4	31.3 – 39.6
8 – 14	77	15.1	11.9 – 18.1
15 – 28	60	11.7	8.9 – 14.5
> 28	67	13.1	10.1 – 16.0

Although there was no difference between TSDG and non-TSDG farms, the numbers of calves within groups on individual farms ranged widely, with 75% of farmers reporting that calves were kept in groups sized between 2 and 7 calves, the remainder of farms housed calves in larger groups (Table 2.8). The age difference of calves within groups was usually no more than 2 weeks (*n* = 374, 78.9%, 95% CI 70.6 - 78.9) with a small number of farms grouping together pre-weaned animals that were more than 4 weeks different in age (*n* = 31, 6.5%, 95% CI 4.3 - 8.8).

Table 2.8: Number of calves in groups during the pre-weaning phase of life for both TSDG and non-TSDG farms.

Number of calves per group	*n*	% of farms	95% Confidence interval
2 – 4	179	36.8	32.5 – 41.0
5 – 7	186	38.2	33.9 – 42.5
8 – 10	77	15.8	12.6 – 19.1
> 11	45	9.2	6.7 – 11.8

Straw was the bedding of choice on most farms (*n* = 692, 98.2%, 95% CI 97.2 - 99.2), with 322 farmers (46.1%, 95% CI 42.4 - 49.8) reporting that they removed and replaced bedding when one group of calves left and a new batch entered. Six hundred and sixteen farmers (85.2%, 95% CI 82.6 - 87.8) reported that they disinfected calf pens or housing, with this task being performed between groups of calves on 309 (51.2%, 95% CI 47.2 - 55.2) farms.

Calf Feeding

Again, whilst there was considerable variation between individual farms, there were no significant differences in calf feeding practices between TSDG and non-TSDG farms ($P < 0.05$), with the exception of the use of dummy teats.

Prior to weaning, 397 farmers (55%, 95% CI 51.3 - 58.5) reported that they fed milk replacer to their calves, with 450 (62.2%, 95% CI 58.7 - 65.8) reporting that they fed waste milk, although not always exclusively. Combining the responses to the above questions would suggest that whilst 179 (24.8% 95% CI 21.6 - 28.1) farms fed both milk replacer and waste milk, 218 (30.1% 95% CI 26.8 - 33.6) farms fed milk replacer only and 271 (37.5% 95% CI 34 - 41.1) farmers reported that they fed waste whole milk only. Two hundred and three (28.1%, 95% CI 24.8 - 31.4) farms reported that they feed pooled colostrum or pooled milk to calves.

Three hundred and fifty (50.0%, 95% CI 46.3 - 53.7) farmers reported that they fed calves from individual buckets, 223 (31.9%, 95% CI 28.4 - 35.3) reported feeding milk from troughs whilst computerised milk feeders were used on only 53 (7.6%, 95% CI 5.6 - 9.5) farms.

Whilst few farmers (*n* = 79 10.9%, 95% CI 8.6 - 13.2) reported allowing calves access to a dummy teat, TSDG farmers were significantly more likely (P < 0.001) to use dummy teats (*n* = 70 13.6%, 95% CI 10.6 - 16.5) compared to non-TSDG farmers (*n* = 9 4.3%, 95% CI 1.6 - 7.1).

The *ad libitum* feeding of milk or milk replacer occurred on only 48 (6.6%, 95% CI 4.8 - 8.5) farms. The majority of farmers (*n* = 537 87.0%, 95% CI 84.4 - 89.7) reported that they fed calves twice per day, whilst 35 farmers (5.7%, 95% CI 3.8 - 7.5) only fed milk once daily. A further 39 farms (6.3%, 95% CI 4.4 - 8.2) fed calves *via* a computerised automatic feeder such that the number of meals received daily was unknown. Only 6 (0.9%, 95% CI 0.1 - 1.7) farmers reported feeding calves three times daily.

Whilst the majority of responders (*n* = 355 58.9%, 95% CI 54.9 - 62.8) reported that they fed between 2 - 3 litres at each feed, 148 farmers (24.6%, 95% CI 21.3 - 28.2) reported feeding between 3 - 5 litres at each feed, and 99 farms (16.4% 95% CI 13.5 - 19.4) fed less than 2 litres at each feed (Figure 2.6).

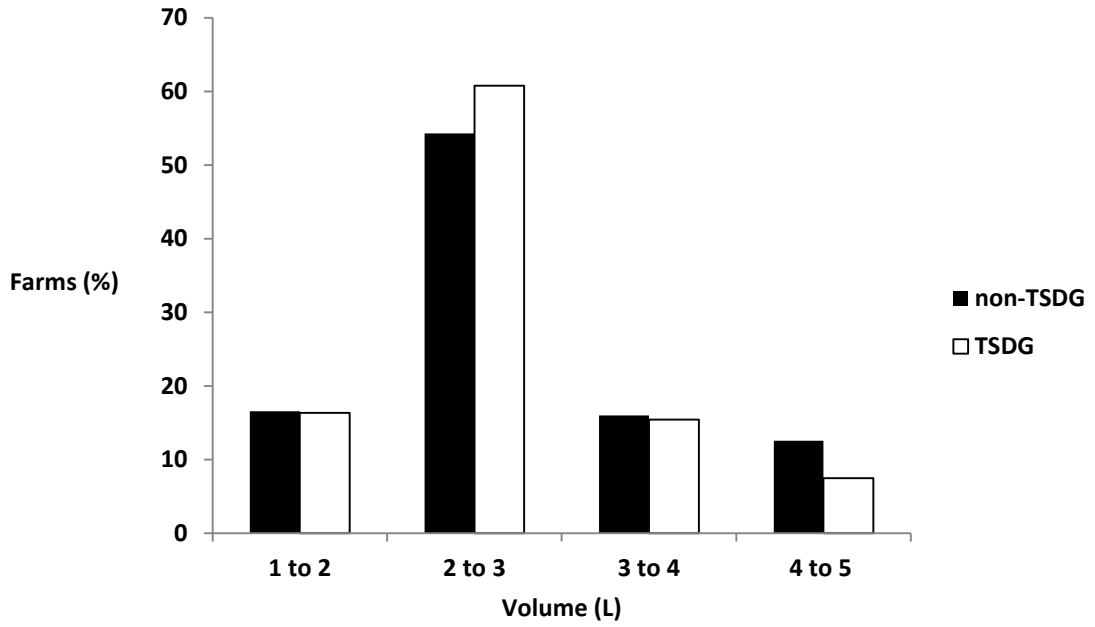

Figure 2.7: Volumes of milk or milk replacer fed to calves during each feed on TSDG and non-TSDG farms.

While there was no association between annual milk yield per cow and number of times calves were fed using a bucket, teat or trough, farms which fed calves *via* a computerised machines reported significantly higher ($P < 0.004$) milk yields (+ 473.8 litres, 95% CI 149.4 - 798.2) compared to farms feeding milk *via* a bucket, teat or trough after adjusting for herd size.

Approximately half of all farmers (*n* = 346, 49.9%, 95% CI 46.2 - 53.7) reported cleaning feeding vessels between each feed, 213 (30.7%, 95% CI27.3 - 34.2) reported cleaning them on a daily basis and very few farmers (*n* = 141, 19.3%, 95% CI 14.9 - 23.8) cleaned them on a less regular basis.

Whilst concentrates were fed by all farms to young calves, there were differences in feeding practices reported. The availability of concentrates varied with access during the first 7 days of life on 363 (52.1%, 95% CI 48.4 - 55.8) farms, between 7 and 14 days of life on 245 (35.1%, 95% CI 31.6 - 38.7) farms and after 14 days on 89 (12.8%, 95% CI 10.3 - 15.3) farms.

The type of concentrate feed being provided to pre-weaned calves was pelleted feed only on 402 (57.5%, 95% CI 53.8 - 61.2) farms and coarse mix only on 215 (30.8%, 95% CI 27.3 -

34.2) farms, whilst a small number of farms provided both pellets and coarse mix as a pre-weaning concentrate feed (*n* = 68, 9.7%, 95% CI 7.5 - 11.9). The majority of farmers (*n* = 525 76.5% 95% CI 73.4 - 79.7) reported that concentrate feed was provided on an *ad libitum* basis.

Farmers reported that forage was offered to calves in the first week of life on 331 (47.9%, 95% CI 44.2 - 51.6) farms, with 197 (28.5%, 95% CI 25.1 - 31.9) reporting that forage was offered between 8 and 14 days. There were 163 (23.6%, 95% CI 20.4 - 26.8) farms that did not offer forage until over 2 weeks of age. The type of forage offered varied between farms, with almost half (*n* = 342 48.6%, 95% CI 44.9 - 52.4) offering straw, 204 (29.0%, 95% CI 25.7 - 32.4) offering hay whilst a small minority (*n* = 33 4.7%, 95% CI 3.1 - 6.3) offered silage. A small number (*n* = 75 10.7%, 95% CI 8.4 - 13.0) stated that they offered no forage at all but expected calves to eat their bedding as a forage source.

Fresh water was given to calves on most farms (*n* = 672 92.9%, 95% CI 91.1 - 94.8) and there was a wide variation regarding the frequency of water changing, with 83 (11.5%, 95% CI 9.2 - 13.8) farms not changing water on a regular basis.

Routine Medication and Weaning

Only 113 (15.6%, 95% CI 13.0 - 18.3) farms reported that they administered routine medication to calves with no significant difference between TSDG and non-TSDG farms (P = 0.150). Medication used was mainly for Coccidiosis (*n* = 39, 5.4% 95% CI 3.7 - 7.0), *Cryptosporidium* (*n* = 15, 2.1%, 95% CI 1.0 - 3.1), and respiratory diseases (*n* = 24, 3.3%, 95% CI 2.0 - 4.6), with only four farms (0.6%, 95% CI 0.01 - 0.1) reporting that they administered antibiotics in milk or milk replacer.

There was considerable variation between farms regarding variables used to determine the onset of weaning of calves from milk or milk replacer to a solid diet. Four hundred and fifty one (62.4%, 95% CI 58.8 - 65.9) farmers reported that they used age of calf as the main criterion as to when to wean, whilst 406 (56.1%, 95% CI 52 - 60) reported that they used concentrate intake to determine weaning age. There was a marginal difference between TSDG and non-TSDG farmers in their response to this question with significantly more non-

TSDG farmers (P = 0.051) using concentrate intake as a criterion for weaning (*n* = 128 61.8%, 95% CI 55.2 - 68.5) compared to TSDG farms (*n* = 278 53.9%, 95% CI 49.6 - 58.2). Body size was also reported as being used as an indicator of when to wean calves on 407 (56.3%, 95% CI 52.7 - 59.9) farms, with no difference between TSDG and non-TSDG farms. There was considerable variation in the age at which farmers reported weaning their calves (Table2. 9).

Table 2.9: Age at weaning for calves on farms within the study.

Age at weaning (weeks)	***n***	**%**	**95% Confidence interval**
< 6	80	12.0	9.6-14.5
6 - 8	317	47.7	43.9-51.5
8 - 10	160	24.1	20.8 – 27.3
>10	108	16.2	13.4 – 19.1

Very few farmers (*n* = 7, 0.9%, 95% CI 0.3 - 1.7) reported that they undertook any routine weighing or measuring of calves in order to assess growth.

2.4 Discussion

The aim of this study was to determine current U.K. dairy calf rearing practices and to identify any differences between farms bound by a Supermarket contract (TSDG) and those that were not.

Members of the TSDG were paid 31.58p per litre, compared to a non-Supermarket contracted farmer who received between 29.00 and 29.50p per litre of milk (October 2012). In 2011 Tesco developed and rolled out a new Code of Practice (COP) for dairy farmers, which specified that key performance indicators such as lameness scoring, antibiotic usage, vaccination and environmental measures were to be recorded by each farm on a regular basis. There are 17 absolute measures and 15 measures included in the new COP, by which improvements to herds can be made. Farms within the TSDG are able to benchmark themselves against other farmers within the same group.

The response rate for this questionnaire study was excellent, with 72% of questionnaires being returned via the milk processors who sent the questionnaires out on behalf of the authors. The high response rate was most likely due to the fact that farms were contracted by the milk processors and were therefore wary of any perceived penalties that may be imposed for non-response.

Data from this questionnaire provides the most up to date information regarding dairy calf rearing strategies within the U.K. Although the response rate was very high, it must be remembered that the accuracy of some of the responses may be poor. In fact, there were a number of discrepancies in answers to certain questions, especially those regarding milk feeding practices. This was accounted for in the analysis by categorising and/or deleting certain questions. With all studies of this nature, it is impossible to acquire completely accurate information when human opinion is involved (Pennings et al., 2002). Some of the questions were not answered fully and in many circumstances farmers chose not to divulge the information asked for by offering vague answers.

Data gathered for staff employed on farms suggests that there is no difference between TSDG and non-TSDG farms in numbers of workers, with the majority of staff having at least 10 years experience in calf rearing. As would be expected many farms have different people

looking after calves at the weekend which could impact on health and welfare through unfamiliarity with feeding protocols and individual animal needs. It is interesting to note that there appeared to be a trend that more TSDG farms had calf staff with less than 1 years experience than non-TSDG farms. This may reflect increased confidence in the sustainability of dairying amongst TSDG members and they may be more likely to invest in training the next generation. The TSDG also encourages knowledge exchange and there may be a larger number of students or work experience pupils on TSDG farms gaining an important insight into the dairy industry.

Dairy herd size was significantly larger on TSDG farms compared to non-TSDG farms. This may be a reflection of selection for TSDG membership or, that farms within the TSDG have been able to expand their herd size due to the increased financial support from a higher milk price. TSDG farms had a lower proportion of youngstock (compared to adults) on their farms than non-TSDG farms. This may be due to less 'wastage' of animals during the rearing period on TSDG farms compared to non-TSDG farms, such that less youngstock need to be reared to supply replacement animals. Alternatively, it may be that non-TSDG farms sell more youngstock than TSDG farms since they are not expanding their businesses at the rate that the TSDG group are able to. No questions were asked regarding morbidity or mortality since it is well recognised that the majority of farmers are poor in recording youngstock health events (Robb, 2006); however, the data gathered in this study on youngstock rearing may suggest that youngstock mortality is lower on TSDG farms. Brickell *et al* found considerable variation in youngstock morbidity and mortality on U.K. farms (Brickell et al., 2009).

In this study, the mean cull rate for adult cows was 19.5% with no difference between TSDG and non-TSDG farms. Esslemont *et al.* reported a mean cull rate of 23.8% (Esslemont and Kossaibati, 1997), whilst current estimates of cull rate in the U.K. are between 18 and 35% (DairyCo.), the data gathered in this study is in agreement with these figures.

Over one third of farms in this study claimed to be of closed herd status which in terms of biosecurity provides excellent protection against introduction of some infectious diseases (Brennan and Christley, 2012). There were only a very small number of farms (4.1%) that operated on a flying herd system. Such systems are entirely focused on the adult milking

cow and not on rearing youngstock; this system is at a high risk of introduction of infectious disease to the herd population.

Eighty nine percent of all farms kept Holstein-Friesian cows. This finding was expected as this breed is genetically the highest yielding, favouring maximal profit and reduced costs of production. Mean milk production was 8244 litres per cow annually. This is higher than the U.K. average for 2010 which was 7315 litres: this is in agreement with a trend for increasing milk yields in recent years (House of Commons Library).

There was a positive correlation between milk yield and herd size, this perhaps illustrates how larger herds tend to be more efficient and are more able to produce a higher yield per cow due to improved management techniques, improved diet formulation and more specific grouping of animals.

It has long been documented that fertility rates within Dairy herds have decreased significantly since the 1960's when conception rates were as high as 60%. It is currently estimated to be falling at a rate of 1% per annum, such that it is now as low as 20% on some farms (Royal and Flint, 2004). This precipitous decline in fertility has a major economic impact on the dairy industry as a whole. However, there has been some evidence that this decline in fertility is halting with the implementation of the fertility index for bull selection in dairy herds (DairyCo, 2014).

Traditionally, a calving index of 370 – 380 days has been considered optimal, however this is rarely achieved in UK dairy herds (Esslemont and Kossaibati, 1996). Currently the average calving index in the UK is estimated at being greater than 410 days (DairyCo. 2014).

Whilst mean calving index was 407 days in this study, it was significantly shorter by 6 days on non-TSDG farms. However, there were many farmers who did not answer this question which may introduce bias of unknown direction. This failure to answer may be due to poor record keeping leading to an unknown average calving index, or alternatively that farmers recognised that their calving index was unacceptably high and did not wish to publish the data i.e. responder bias (Pennings et al., 2002).

There was a positive association between calving index and milk yield which supports the hypothesis that declining fertility is associated with increased milk yield (Royal and Flint,

2004; Wathes et al., 2008). The risk for metabolic disease is greater in high yielding animals due to the large pressure presented to them. High milk production promotes insulin resistance in these animals which, in turn, will reduce fertility rates and therefore increase calving indices.

Calving index, although a useful indicator, may not give the whole picture in terms of herd fertility. The lower calving index value recorded on non-TSDG farms compared to TSDG may have been due to culling of individual animals with high calving indices, they would therefore not appear in the calving index data. As expected, cull rates for heifers was much lower than that of adult cows, the majority of deaths or culls in young animals is due to accidents rather than to less than optimal performance at this age (Brickell et al., 2009).

The breeding policies on farms varied, with most farms utilising both artificial insemination and bull service (64%) with no difference in policy between TSDG and non-TSDG farms. Heifers were served with dairy bull semen only in 40% of cases and with dairy or beef semen in a further 41% of cases. This high use of beef sires represents a lost opportunity in terms of herd level genetic improvement. The fertility rate of heifers is often higher than that of cows due to reduced metabolic pressure on these animals to produce optimal milk yields whilst maintaining a pregnancy and selection of sire is most likely based on this (Taylor et al., 2003).

Vaccination of adult cows was commonplace on the majority of farms and usage, irrespective of type, was associated with higher annual milk yield per cow. Whether this was a direct effect of vaccine usage improving herd health and thereby milk yield, or was a proxy indicator of “good farm management”, it cannot be ascertained from this data.

There were very few farms that vaccinated for lungworm. Traditionally, lungworm is perceived as a disease of the first grazing season with challenge at this stage ensuring lifelong immunity (Blowey, 1999). Control involves either vaccination, which is considered to be the ideal method, or ensuring that animals have periods of natural exposure to infected pastures followed by strategic anthelmintic treatment. In recent years, there has been an increase in adult cases of lungworm which is thought to be attributable to falling vaccine usage and an increased reliance on anthelmintics (Coles et al., 2010). These data suggest

that a considerable number of youngstock may not be acquiring protective immunity rendering them at risk of lungworm infection as adults.

Calf vaccination was less frequent than adult vaccination irrespective of membership of TSDG, with less than half of all farms vaccinating young animals. As was the case with adult vaccination there was a positive association between calfhood vaccination and annual milk yield per cow. The reasons behind this apparent association are unclear but it may be that as with adult vaccination, calfhood vaccination is a proxy indicator of "good farming".

Whilst there was no significant difference in annual milk yield per cow between TSDG and non-TSDG farms, there was a trend for more non-TSDG farmers to describe their enterprise as being "low input: low output". It could be argued that TSDG farmers may be more inclined to invest in their businesses by virtue of the financial security offered with their contract with the retailer. There is however, an alternative explanation that Supermarkets are less inclined to recruit low input: low output farms, since such grass based systems tend to have a strong seasonal production bias with peak yields in the summer months and minimal or no production in winter. This is undesirable from a retailer's point of view since a constant supply of fresh milk is required throughout the year with no seasonal peaks or troughs.

Sixty seven percent of farms studies did not allow their youngstock access to pasture in the period up till first calving. This is in contrast to a study carried out by Boulton *et al.* whereby 95% of farms included in a questionnaire based U.K. dairy farm study turned their youngstock out to pasture prior to first calving (Boulton et al., 2015). The disparity between studies is unclear but may be a reflection of different study populations.

Over 90% of farmers housed adult lactating cows in cubicles with the remainder housed in straw yards. Whilst over half of farmers reported housing dry cows in cubicles, there was a trend for cows to be housed in straw yards during the last 3 weeks of pregnancy. This affords greater comfort albeit at the increased risk of acquiring environmental mastitis pathogens (Fregonesi and Leaver, 2001).

There was considerable variation in housing and management of the calving cow between individual farms, although there was no association with TSDG membership. Whilst all

calves were born inside during the winter months, half of farmers reported calving cows outside during the summer months. This has obvious benefits to the calf in terms of reduced pathogen exposure compared to indoor grouped calving (DEFRA, 2003).

Whilst over half of farmers stated they had individual calving boxes on their farms, only 30% used them at all and only 21% stated that they only calved cows in individual boxes. The majority (78%) of farmers reported having between 1 and 4 calving boxes which would be considered insufficient on the majority of holdings. This together with the increased labour demands in terms of observing cows and moving them to boxes to calve is likely the reason for the low uptake reported here.

The majority (66%) of farmers calved cows in group housing with almost half of these reporting that there were no dedicated calving facilities on these farms. This management system has increased in popularity with increasing herd size and labour constraints. Whilst this practice may be less stressful for the cow, it undoubtedly increases the risk to the calf of acquiring faecally borne pathogens such as enteric viruses and protozoa, which may result in neonatal disease. There is a high probability that calves born in such circumstances may become infected with *Mycobacterium avium* subsp *paratuberculosis*, the cause of Johne's disease in later life.

The level of pathogen challenge to the newborn calf will depend on the hygiene in the calving area, which is dependent on stocking density, bedding quality and the time period for which the calf is exposed to the calving environment. Traditionally, a calf will be left with it's dam for between 24 - 48 hours after birth to allow maximal colostrum intake and reduce stress on both the cow and calf (Viera et al., 2011). However it is now recognised that leaving the calf for extended periods with its dam has little impact on colostrum intake, and current best practice is to remove the calf from its dam within 2 hours of birth (Mee, 2008) in order to minimise pathogen acquisition during this period. Whilst almost half of farmers reported removing the calf within 24 hours of birth, only 6% stated that calves were removed within 3 hours of birth suggesting that few farmers are unaware of current best practice or are not in agreement with it.

The importance of sufficient colostrum consumption in the first few hours of life to provide protection against inevitable pathogen challenge is well recognised (Berge et al., 2009;

Besser et al., 1991; Chigerwe et al., 2008; Godden, 2008; Morin et al., 1997; Smith and Little, 1922; Weaver et al., 2000). Current best practice is not to rely on natural suckling by the newborn calf but to administer 3 -4 litres via a bucket, teat or stomach tube within 6 hours of life (Chigerwe et al., 2008; Cortese, 2009; DEFRA, 2003; McGuirk, 2007).

There was considerable variation in colostrum feeding practices on farms in this study irrespective of TSDG membership. The majority of farmers (52%) allowed natural sucking and only administered colostrum if they thought it was necessary. Almost as many (41%) reported that they did administer colostrum to all calves whereas a minority (5%) did not administer colostrum (allowed natural sucking only). Time of birth (night or day) had a significant effect on timing of colostrum administration with calves more likely to receive colostrum earlier if born during the day. Over half (55%) of all farmers stated they ensured calves received colostrum within 6 hours of birth irrespective of time of birth. These data would suggest that a large proportion of calves on UK dairy farms are not being managed in such a way as to optimise colostral antibody absorption. This is in agreement with numerous studies showing significant numbers of dairy calves with sub-optimal serum Ig concentrations (Robison et al., 1988; Weaver et al., 2000; Wells et al., 1996).

Whilst the majority of farmers (88%) administered the dam's own colostrum to newborn calves, a small minority (5%) administered colostrum pooled from multiple cows, this is a well recognised risk factor for transmission of Johne's disease (Nielsen et al., 2008).

Correct housing conditions for young calves are key to minimising morbidity and mortality (Wathes et al., 1983). Over 80% of farms in this study housed neonates in designated barns or houses. A small proportion of farms housed young calves in buildings that were shared with older animals. This is not ideal due to exposure of neonatal animals to high pathogen loads via the "pathogen multiplier effect" (Blowey, 1999) where older animals sharing the same air space during a period in which the immune system is not yet fully developed will increase the risk of infection to neonates. There was no significant difference between TSDG and non-TSDG farms in this respect (although a higher percentage of TSDG farms used shared buildings than non-TSDG). It is likely that this practice of using "shared buildings" is due to lack of designated facilities on these premises. Individual pens were used for pre-weaned calves by 45% of farmers, with 63% reporting that they group housing animals at

some point during the pre-weaning period. Individual penning can offer protection against spread of contagious disease through physical contact and is used on many farms for this reason. The ability to determine the feed and milk intakes of calves housed individually is much easier than that of group housed animals, but this may be at the cost of the welfare of the calf. Careful group housing of young calves within a similar age range can be beneficial in terms of reduced labour costs (Kung et al., 1997) and improved animal welfare. The ability for young calves to display natural play behaviour and to be able to move around freely is important (De Paula Vieira et al., 2010; Jensen and Kyhn, 2000). There is evidence to show that group housed calves have an increased intake of concentrate feed at an early age compared to that of individually housed calves (Warnick et al., 1977).

The use of hutches for pre-weaned calves was recorded by 12% of farmers. This housing system allows calves access to both inside and outside areas resulting in improved health and welfare due to increased space compared to an individual pen, along with superior air quality (McKnight, 1978). Disadvantages of this system include the increased area taken up by the use of hutches, extremes of temperatures and harsh conditions for farm workers in bad weather.

The thermo-neutral zone for a young calf is much narrower than that of an older animal (Lago et al., 2006) due to the higher surface area to volume ratio and the inability of young calves to thermoregulate as efficiently, thus leaving them highly susceptible to hyper or hypothermia. Newborn calves have limited quantities of brown adipose tissue available for use as a thermoregulatory 'organ' during the first few weeks of life, however this is in relatively short supply (Carter and Schucany, 2008). Potential hypothermia resultant on environmental conditions is accentuated by negative energy balance which is commonplace in young calves (less than 2 weeks of age) due to restricted milk or milk replacer feeding (McGuirk, 2007).

Farms that group housed calves did so at various ages; most (35%) did so at between 3 and 7 days and the age range between groups was no more than 2 weeks on 78% of farms. Grouping animals of similar age and size is important for prevention of disease and to avoid bullying of small calves by bigger older calves, which may result in sub-optimal food intakes (Færevik et al., 2010).

Traditional methods of dairy calf feeding involve restricting volumes of milk or milk replacer offered and to weaning onto concentrate feed as early as possible. Along with cost-saving advantages, the justification for this method has been that rumen development will be promoted at an earlier age than in predominantly milk fed calves (Coverdale et al., 2004). It is believed that this will result in a larger rumen size in the adult animal, thereby allowing maximal DMI and milk production, in recent years this strategy has come under increasing scrutiny (FAWC, 2004). Welfare issues associated with restricted milk feeding have also come to the forefront of research, and have cast doubt on the restricted method of pre-weaning feeding (James, 2008; Moallem et al., 2010; Quigley et al., 2006; Soberon et al., 2012). The benefits of increased milk feeding have been studied by various research groups (Anderson, 2011; Appleby et al., 2001; Drackley, 2008; Hill et al., 2013; Jasper and Weary, 2002; Richard et al., 1988) and some farms have adopted extended milk feeding periods or increased milk volumes based on the growing evidence base.

Although most farms in this study fed between 2 and 3 litres of milk per feed and fed calves twice daily (85%), there were a minority (16%) of farms that fed between 3 and 4 litres per feed and a few that fed more. There were only 7% of farms that fed calves *ad libitum* milk or milk replacer. Although there is evidence in the literature that *ad libitum* feeding of milk or milk replacer is beneficial (Bach, 2011b; Drackley; Van Amburgh et al., 2011), U.K. farmers are not, as a whole, adopting this strategy. In this study there was no clear association between average milk yield and amount of milk fed. Although farms that fed calves via a computerised milk feeder had increased milk yields in their adult animals, this was unlikely to be a causal association.

Just over half of farmers reported (55%) feeding calves with milk replacer. This method of feeding ensures continuity and quality of the product fed, and eliminates the risk of disease transmission that is present during feeding of waste milk. There were a large number of farmers (62%) feeding waste milk to calves and many fed both MR and waste milk. Feeding of waste milk reduces the cost to the farmer and makes use of milk that would otherwise be discarded as it would be unfit for sale. However, feeding of waste milk may have adverse effects, such as increased transmission of Johnes disease (Nielsen et al., 2008) and possible implications regarding antibiotic resistance (Langford et al., 2003).

Method of feeding calves also varied between farms with no difference between TSDG and non-TSDG groups. Both teats (45% of farms) and buckets (40% of farms) were used for feeding milk or milk replacer. Cleanliness of feeding vessels was good in most cases as cleaning occurred either between feeds or on a daily basis in 81% of farms. The significantly higher number of TSDG farms (*n* = 70, 14%) that allowed calves access to dummy teats was not surprising as the TSDG code of practice states that all calves should have access to this facility.

The importance of consumption of concentrate feed at an early age for dairy calves is well known (Bach et al., 2010), and calves on more than half of all farms were able to access concentrate feed within the first 7 days of life. Pelleted feed was preferred on 58% of farms over a coarse mix feed. This was an interesting finding as many studies have documented that coarse mix is a more palatable feed to young calves than pellets (Franklin et al., 2003). The cost of pelleted concentrate feed is often lower than multi particle feeds and this may be the reason for the predominant use of pellets in this study. The type of forage offered to calves did not differ between TSDG and non-TSDG groups with most farms offering straw (49%) or hay (29%). Some farms did not offer forage as a feed but just allowed calves to eat their bedding (*n* = 75, 11%) and nearly one quarter of farms did not offer forage to calves until they were over 2 weeks of age. This may present a significant risk for disease via ingestion of disease causing agents contained in faeces (Garber et al., 1994).

Fresh water was offered to calves on 93% of farms with only 89% of farms allowing 24 hour access. The provision of fresh water for dairy calves is a legal requirement in addition to the knowledge that fresh water is necessary for the optimal population of bacteria within the rumen which in turn drives rumen development (Kertz et al., 1984, DEFRA, 2003).

Routine medication of calves was uncommon on farms with a total of 16% of farmers reporting routinely administering medication. Coccidiosis medication was most common and very few farms added antibiotics to milk or MR. Routinely adding antibiotics to calf milk or feeding waste milk containing antibiotics from cows increases the risk of antibiotic resistance (Langford et al., 2003). Public concern over the use of antibiotics in food producing animals has been increased in recent years (Anderson, 2011) and as a group, the

TSDG is working towards educating their farmers and ensuring recording of the use of these drugs occurs on TSDG farms and that antibiotics are only used when necessary.

Measurement of growth and weight only took place on 1% of farms in this study. It is well established that measurement is key to effective decision making (DEFRA, 2003; Development).

In this study, decisions on when to wean calves were based mainly on age (62% of farms) or concentrate intake. More non-TSDG farms used concentrate intake as a criteria for weaning, although the difference between TSDG and non-TSDG farms in this area was only marginally significant ($P = 0.051$). Body size was used as a criterion on over half of farms, this was carried out by eye and was thus a rather subjective measure.

In conclusion, calf rearing practices did not differ between farms that were part of the TSDG and farms that were not. The Supermarket contracted farms did not appear to manage calves any differently, nor did they produce higher milk yields or have lower cull rates. There were highly variable husbandry techniques between farms and varying levels of intensity of farming. Vaccination of adult animals appeared to have the most significant association with annual milk yield per cow rather than a specific management strategy during early life. However, the information gathered from this study must be used carefully to draw conclusions. Postal questionnaires may not yield the most accurate data due to the ability for responder bias.

This study provides the most up to date information on calf rearing strategies used by dairy farmers in the U.K. and has identified areas of strengths and weaknesses in terms of animal husbandry. This information may be used in future to further optimise dairy calf rearing strategies, thereby improving the overall health and welfare of animals on U.K. dairy farms.

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Chapter 3

Effect of milk replacer feeding strategy on the growth and health of Holstein heifer calves: from birth to 12 weeks of age

3.1 Introduction

For optimal lifetime performance and profitability, Holstein dairy heifers should deliver their first calf and enter the milking herd at a maximum age of 24 months. The key to achieving this target is correct nutrition and management so that by 13 - 14 months the recommended target bodyweight of 380 - 400 kg is achieved, accompanied by adequate skeletal growth as demonstrated by a withers height of at least 125 cm (Brickell et al., 2009a; Corbett, 2010; Drackley, 2008; James, 2008; Morrison et al., 2012). Unfortunately many dairy farms fail to achieve these growth targets and suboptimal management of calves and growing heifers is often linked to high disease incidence, which in turn has a huge economic impact on the industry (Brickell et al., 2009b; Waltner-Toews et al., 1986).

In addition to age at entry into the milking herd, the long term health and productivity of these heifers as replacement milking animals is of major importance in terms of profitability and sustainability. A short productive lifespan associated with early culling is a major constraint on the lifetime profitability of the modern Holstein dairy cow, and metabolic disease plays a major role in this (Esslemont and Kossaibati, 1996; Wathes et al., 2008). Studies carried out in humans have shown that malnutrition during very early life can increase the likelihood of metabolic disease during adulthood (Heijmans et al., 2008), and it is hypothesised that a similar situation applies to the modern dairy cow. Whilst recent studies have demonstrated a positive impact of increased milk intake on future milk production (Bar-Peled et al., 1997; Drackley et al., 2007; Soberon et al., 2012; Soberon and Van Amburgh, 2013; Van Amburgh et al., 2011) there is currently scant data on the impact of early life nutrition on health and longevity.

Current pre-weaned dairy calf rearing strategies have focused on feeding milk or milk replacer (MR) at a rate of 10% of body weight daily (De Paula Vieira et al., 2008). Energy availability from such small quantities of milk alone is insufficient to support growth. The underlying rationale for feeding limited volumes of milk or MR are both the relative high cost compared to solid foods, and the belief that restricting milk intake in early life will encourage the calf to consume solid food more rapidly, thus accelerating and improving development of the rumen.

The calf is born with a non-functional rumen and development is in response to intake of grain based feedstuffs which undergo fermentation to produce volatile fatty acids (VFA's).These VFA's (butyrate and propionate) are the main stimulus for rumen papillae development and therefore a functional rumen (Smith, 1958). However, this development takes at least three weeks and so during the early life period the calf will be reliant on liquid milk or MR as the sole energy source. This inability to consume and utilise sufficient concentrate feed at an early age means calves fed restricted milk or MR during the first three weeks of life, have insufficient energy for growth and furthermore are in a state best described as "chronic hunger". Unable to support growth at this level of nutrition, opportunities for development during periods of high feed efficiencies are missed.

Disease in young calves has further negative impacts on growth rates (Gorden and Plummer, 2010), resulting in increased production costs, directly due to necessary disease treatment and indirectly due to increased time to first calving (Waltner-Toews et al., 1986). A recent study of 19 dairy farms in the U.K. demonstrated considerable variation in average growth rates of calves both between farms (range 0.49 - 1.02 kg/day) and within farms (0.45 - 1.13 kg/day) from 1 to 6 months of age (Brickell *et al.,* 2009a). It was hypothesised that the within farm variation was a reflection of disease in the populations studied.

At the current time, the majority of pre-weaned calves are fed twice daily with MR *via* a bucket or teat, the volume of milk offered at each feed varies from 1.5 - 3 litres (Chapter 2). Once daily milk feeding is also practiced by a minority of U.K. dairy farmers, although there is currently concern on the welfare aspects of this practice (van der Burgt and Hepple, 2013). An increasingly popular method of milk feeding allowing delivery of more frequent feeds of specified volumes, is the use of computerised milk feeders. This is believed to better satisfy the behavioural needs of the calf by allowing suckling at the desired time (Appleby et al., 2001; Nielsen, 2012). Similarly a number of producers feed calves on an *ad libitum* basis whereby the calves may consume MR to appetite. Such systems either utilise computerised feeders or feed acidified milk or MR (Anderson, 2008; Hill et al., 2013; Quigley and Bearden, 1996).

Many studies have evaluated the effects of increased milk or MR feeding; results from these studies are unanimous in demonstrating positive effects in terms of growth, health and

welfare (Anderson, 2011; Appleby et al., 2001; Borderas et al., 2009; Drackley et al., 2007; Hill et al., 2013; Jasper and Weary, 2002). However these findings do not yet appear to have had an impact on U.K. dairy calf management practices, with the majority of farmers continuing to feed limited amounts of milk or MR during early life (Chapter 2). This may be due to perceived problems traditionally believed to be associated with increased milk feeding, such as nutritional diarrhoea and reduced concentrate feed intakes (Appleby et al., 2001; Hepola, 2003). Furthermore, there are few studies that have assessed the full lifetime performance of animals given access to increased milk or MR during early life. The majority of the afore-mentioned studies have been carried out in the U.S.A. and there is little detailed work on the impact of early life feeding under U.K. conditions.

The objective of this study was to describe the growth (by measuring body weight and a number of morphometric measures) and health of calves from birth to 12 weeks of age, fed either twice daily with a restricted volume of MR or allowed *ad libitum* access to MR *via* a computerised feeding machine.

3.2 Materials and Methods

The study was performed between January 2011 and January 2013 at the University of Liverpool's Wood Park Dairy Farm, Neston, Wirral, U.K (53°N). The farm milked approximately 170 Holstein Friesian cows with an annual lactation yield of around 10,500 litres on a 3 times daily milking regime. All cows were housed year round apart from during the last 100 days of lactation during which they were allowed out onto grazing during the summer months. All non-lactating pregnant (dry) cows were housed throughout the 8 week dry period. The calving pattern on the farm was described as "all year round" with no seasonal trends.

All healthy, singleton Holstein heifer calves born between January 2011 and November 2012 were enrolled onto the study at birth. Dam identity, parity and ease of calving (Lo´pez de Maturana et al., 2007) were recorded, and a sample of colostrum was collected and stored at 4°C pending analysis. Calves were born into group calving accommodation with between 5 and 15 cows present. If born between 08:00 and 18:00 hours (day time), calves were removed from their mothers within 4 hours of birth and taken to calf accommodation, if born between 18:00 and 08:00 hours (night time), calves remained with their dam for up to 12 hours before being transferred to the calf house. Calves were assigned to one of two MR feeding strategies on arrival at the calf house in alternate groups of ≤ 6, such that each group of calves had an age range of no more than 14 days. Group A; *ad libitum* MR access (*n* = 50) or Group R; restricted MR access (*n* = 50), Table 3.1.

Between 3 and 4 litres of calves own dam's colostrum (collected as soon as possible after birth) was administered to each calf *via* an oesophageal feeder, and given to the calf at the earliest opportunity after birth, together with further, freshly-collected, dam-specific colostrum meals (fed *via* individual bucket) twice daily (2 litres per feed) for four days before beginning MR feeding (96.97% DM, 22.17% crude protein, 19.76% oil, 7.02% ash, ME 21.570 MJ/kgDM, pH 5.96, Blossom Easy Mix, Volac, U.K.). For calves in Group A, familiarisation and training for use of the automatic computerised teat feeder (Vario feeder, Forster Technik, Germany) from which *ad libitum* MR was dispensed began from birth. Calves in this group were able to access MR from birth in addition to the 4 day dam specific colostrum meals. The specific gravity of the initial colostrum meal was assessed using the

sample collected at birth with a Brix refractometer (Animal Reproduction Systems, CA, U.S.A.). Milk replacer powder was thoroughly mixed with water (125g MR/litre, 37^0C) immediately prior to feeding. The age and timing of weaning from MR differed between the 2 dietary groups (Table 3.1, Figure 3.1).

Milk replacer intakes for individual calves in the group A were recorded daily from the computerised feeder (± 0.1litres). Concentrate feed (Primestart coarse mix, 86.2% DM, 18% crude protein, 8% crude fibre, 9.5% ash, 3.5% oil, ME 14.459 MJ/KgDM, BOCM Pauls Ltd U.K.) intakes were recorded for a subset of calves in both dietary groups between April and September 2012 (*n* = 9 Group A*, n* = 7 Group R). Concentrate intake was determined as mean intake for the group, not on an individual basis.

Table 3.1: Nutritional and husbandry protocols used for all calves in the Group A (*ad libitum* MR access) and Group R (restricted MR access).

Group	**Milk Replacer allowance**	**Milk Replacer feeding method**	**Weaning Protocol**	**Housing Method**	**Concentrate and Forage**
A	*Ad libitum* access until day 63	Automatic teat feeder	Stepwise restriction of daily MR allowance over 21 days	Group housed from birth (n≤6)	*Ad libitum* access to grass hay and 2.5kg concentrate feed (coarse mix) daily
R	5L daily until day 21, then 6L daily until day 56 (provided as 2 equal meals, (09:00 & 17:00hrs)	Individual bucket to day 21, thereafter group trough fed	50% reduction of MR allowance over 7 days	Individually housed until 21 days then group housed (n ≤ 6)	*Ad libitum* access to grass hay and 2.5kg concentrate feed (coarse mix) daily

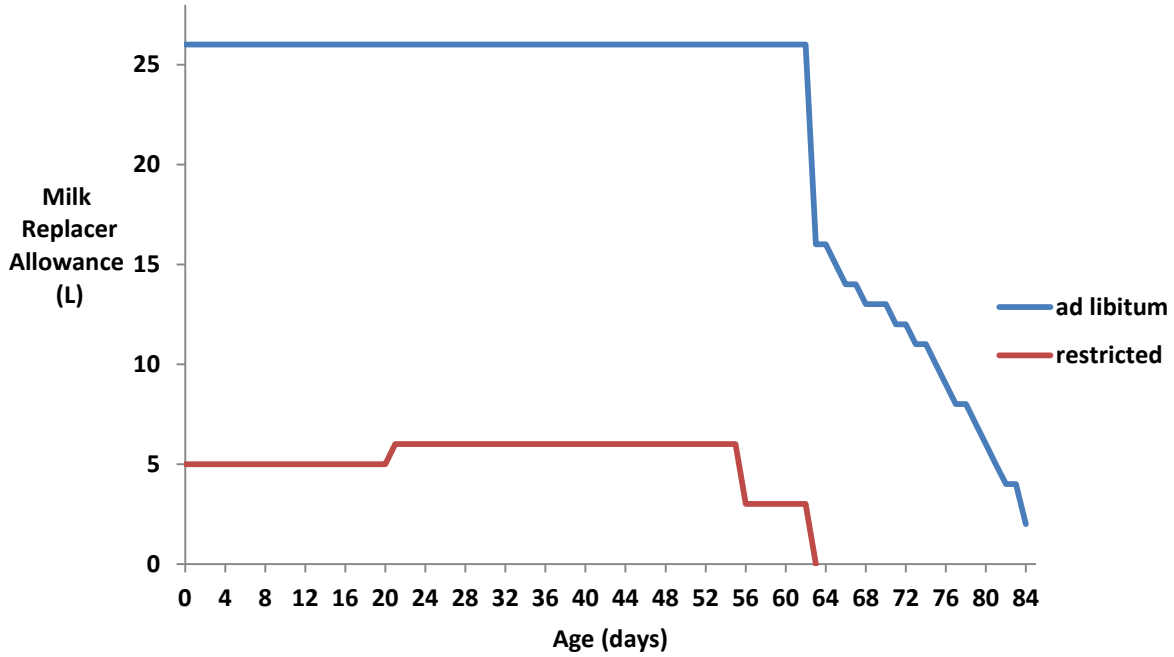

Figure 3.1: Milk replacer allowance (litres/day) for calves in Group R (red line, *n* = 50) and Group A (blue line, *n* = 50) from birth to weaning.

Housing

Calves in Group R were housed individually in metal gated pens (1m x 2m) over raised slatted flooring and bedded with wheat straw from birth until 21 days of age. At 21 days of age, Group R calves were moved to deep wheat straw-bedded group pens (5m x 6m) ($n \leq 6$, age range ≤ 14 days). Calves in Group A were grouped by age (range ≤ 14 days, $n \leq 6$) and were directly introduced to group pens on entry into the calf house. All calves had *ad libitum* access to forage (grass hay), fresh water and coarse mix concentrate feed, up to a maximum of 2.5 kg per head daily.

Measurements

All procedures were performed under the U.K. Animals (Scientific Procedures) Act, 1986. The body weight of each calf was recorded within 12 hours of birth (Ritchey Ltd, North Yorkshire, U.K., ± 0.5kg). During the following 36 hours, measures of the height at the highest point of the withers and loin (±0.1cm, wooden measuring stick, I&D Smallwood, U.K.), circumference of the heart girth (immediately caudal to the elbow) and belly girth (widest part of the belly) (± 1cm), crown to rump length (CRL) (± 1cm) and hock-fetlock length (HFL) (± 1cm, plasticised tape measure) (Table 3.2, Figure 3.2) were recorded and body condition score (BCS) was recorded in accordance with the system presented by Edmonson *et al* (Edmonson et al., 1989) for every calf. During this measurement session a blood sample (20ml) was collected by jugular venipuncture into plain and heparinised 10ml vacutainers (Beckton Dickinson & Son Ltd, Oxford, U.K.). Plasma total protein concentration (PTP) was estimated by refractometry (Clinical refractometer, Hayes, U.K.) and packed cell volume (PCV) was measured *via* centrifugation of heparinised blood in a micro-haematocrit tube at 12000 rpm for 4 minutes. The remaining samples were centrifuged at 2000 x *g* for 15 minutes, aliquoted (plasma and serum) and stored at -20°C.

Body weight was recorded daily from birth until 2 weeks of age and thereafter was recorded on a weekly basis. All morphometric and BCS measures were repeated weekly to 12 weeks of age. Any illness or treatment required by individual calves was recorded. Illness was recorded as either diarrhoea (loose/watery for > 1 day) or pneumonia (when nasal or occular discharge or coughing was noted, or respiratory rate was increased and rectal temperature was elevated; > 39.5°C). All calves presenting with neonatal diarrhoea received 2 litres of an oral replacement solution twice daily (Effydral, Pfizer Ltd, U.K.). All cases of pneumonia were treated with 0.5 mg/kg of meloxicam (Metacam, Boehringer Ingleheim Ltd, Germany) and 2.5 mg/Kg of tulathromycin (Draxxin, Zoetis Ltd, U.K.). A faecal sample was obtained from 3 calves presenting with neonatal diarrhoea in order to identify the causative agent.

Ambient temperature and humidity at the centre of the calf house was recorded daily, approximately 3 feet above floor level, using a minimum-maximum thermometer/hygrometer (Brannan, U.K.). For calves less than 21 days of age, calf 'jackets'

were fitted when the ambient temperature of the calf house dropped below 10°C (Dairy Tech Inc, U.S.A., Figure 3.3).

Statistical Analysis

All data were initially entered into an Excel spreadsheet (Microsoft Corp, USA) and exported to STATA 13 (StataCorp, Texas, USA) for analysis.

Calves at Birth: Simple univariable analyses using linear regression and Students t tests were carried out initially to investigate possible associations between the measured variables. Outcome variables of interest were birth date, birth weight, colostrum quality and plasma total protein concentration at 48 hours.

Calves from birth to 12 weeks: Daily changes in measured parameters recorded over the first 12 weeks of life were calculated for the following time periods for calves in both groups: Birth to 2.99 weeks of age, 3 to 5.99 weeks, 6 to 8.99 weeks and 9 to 11.99 weeks of age. Students t tests were used to compare the mean measurements at different time points between calves in Group A and Group R.

Following simple univariable regression analyses, multivariable regression models were fitted with birth weight (for calves at birth) or morphometric measures (for calves from birth to 12 weeks) as outcome variables.

Measurements were clustered within calves and calves were clustered within groups, therefore 2-level random effects linear regression models were fitted using backward stepwise selection. Likelihood ratio testing for selection of final explanatory variables was carried out. Random effects at both the intercept and slope level were included in all models. The following explanatory variables were offered to the initial models: *Animal factors;* dietary group (restricted MR versus *ad libitum* MR) with an interaction with age (in weeks), dam parity, PTP concentration, presence of “illness” at any stage in the pre-weaning period, age at first colostrum feed and volume of first colostrum feed.

Environmental factors; minimum temperature, minimum humidity, temperature range, humidity range.

Time was included in models to assess seasonal effects as a composite of four sine and cosine functions (Stolwijk et al., 1999). Four time covariates (x_1 x_2 x_3 x_4) were generated: $x_1 = \cos(2\pi t/365.25)$, $x_2 = \sin(2\pi t/365.25)$, $x_3 = \cos(4\pi t/365.25)$, $x_4 = \sin(4\pi t/365.25)$ where t = day with day 1 being the first sample date.

The four sine and cosine time functions were forced into all models to adjust for seasonality, if present. Interaction terms were only included if biologically plausible and retained if they improved model fit as judged by likelihood ratio testing.

Disease: Incidence of diarrhoea and pneumonia were recorded as binary variables. Univariable and multivariable random effects logistic regression models were fitted to investigate associations between putative risk factors and occurrence of disease. Model fitting was carried out using a backward stepwise method as described earlier. Explanatory variables considered for inclusion were dietary group, dam parity, colostrum quality and plasma TP.

Table 3.2: Morphometric measures taken on a weekly basis for all calves in both dietary groups during the first 12 weeks of life.

Variable	**Description**
Body weight	In kg (± 0.5)
Withers height	At highest point of withers (± 0.1 cm)
Loin height	At highest area of loin (± 0.1cm)
Crown-Rump Length	From poll to tail head (± 1cm)
Hock-Fetlock length	From point of hock to fetlock (± 1cm)
Belly girth	Circumference of widest part of belly (± 1cm)
Heart girth	Circumference of area just behind elbow (± 1cm)
Body condition score	Mean value taken from Holstein cow scoring system (Edmonson et al., 1989) in the absence of a calf specific scoring sytem

Table 3.3: Potential explanatory variables for inclusion in multivariable statistical analyses from birth to 12 weeks.

Variable	**Type**	**Description of coding of variable**
Birth weight	Continuous	Weight at birth (kg)
PTP	Continuous	Plasma total protein concentration at 2 days of age (g/L)
Colostrum quality	Continuous	Quality of 1st colostrum (%, Brix refractometry)
PCV	Continuous	Packed cell volume at 2 days of age
Volume consumed in first colostrum feed	Continuous	Volume of colostrum (L) consumed in first colostrum feed
Concentration of Ig consumed in first feed	Continuous	Concentration of immunoglobulins consumed in first colostrum feed (g/L)
Dam parity	Binary	0 = heifer, 1 = cow
Date of birth	date	
Bull	categorical	Identity of bull sire
Gestation	Continuous	Gestation length of the dam
Dietary group	Binary	0 = Group R, 1 = Group A

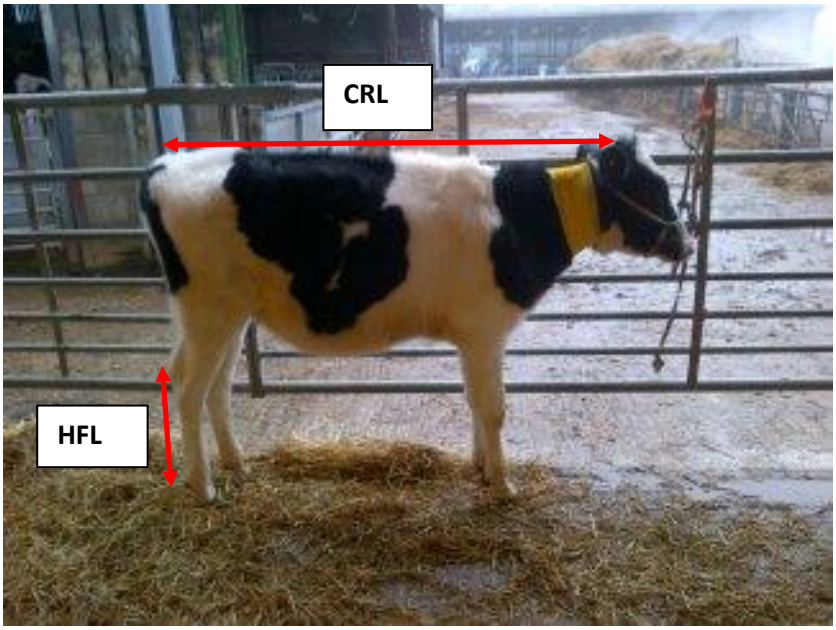

Figure 3.2: Position of measurement for the Crown- rump length (CRL) and Hock-fetlock length (HFL) of study calves.

Figure 3.3: A 7 day old calf fitted with a jacket.

3.3 Results

The study farm operated a year round calving pattern which resulted in births during each month of the year (Figure 3.4).

Overall, the mean gestation length of study calves was 277 days (range; 249 - 290 days). Gestational length varied considerably both within and between sires (Bluesky: mean 284.8 days, S.D. 4.12; Specialist: mean 272.0 days, S.D. 11.3, Table 3.4) and dam parity (primiparous: 274 days, range 249 - 285 days; multiparous: 280 days, range 263-290 days. P = 0.120, Figure 3.5) but was similar for calves in both dietary groups.

Calves at Birth

Overall (*n* = 100), the mean birth weight was 41.78 kg (95% CI, 40.66 - 42.90). However, birth weights were positively associated with dam parity (primiparous: *n* = 43, mean 38.4 kg, 95% CI, 37.1 - 39.8; multiparous: *n* = 57, mean 44.3 kg, 95% CI, 42.9 - 45.7, P < 0.001).

Explanatory variables remaining in the final multivariable model for birth weight were: gestation length, dam parity (primiparous or multiparous) and date of birth (transformed into four sine and cosine functions). Bull identity was included as a random effect since calves were clustered within bull identity (Table 3.6).

Season of calving (date) influenced calf birth weights. After adjusting for other covariates (dam parity, gestation length and bull), summer-born calves were predicted to be 2 kg heavier than those born in winter (Figure 3.6). Dam parity had a significant impact (P <0.001) on birth weight with calves born of multiparous dams being predicted to be 5.2 Kg (95 %CI 4.5 - 5.9) heavier than those from primiparous dams. Birth weight was predicted to increase by 61g (95% CI 32 - 90g) per additional day of gestation. The residual variance that could be attributed to the calf's sire was 22.4% (Table 3.5).

Time from birth to first colostrum ingestion was similar for calves in both dietary groups (overall mean; 3.28 hours, range; 0.25 - 11 hours, P > 0.05). The specific gravity of *peri-partum* colostrum was comparable between dietary groups (23.5%, 95% CI 22.5 - 24.5) and was not influenced by dam parity. Calves born to primiparous dams consumed less

colostrum in their first meal (primiparous: 2.96 L, 95% CI 2.77 - 3.15 L; multiparous: 3.26 L, 95% CI 3.11 - 3.40 L, P = 0.006). However, when data were normalised for calf body weight, initial colostrum intakes were similar for all calves (primiparous dams: 0.077 litres/kgBM; multiparous dams: 0.075 litres/kgBM, P = 0.337).

Serum and plasma total protein (PTP) concentrations were highly correlated (*n* = 100, r = 0.930) and plasma-derived values were used for subsequent analyses. Forty-eight hour PTP concentrations were similar for calves between dietary groups and dam parities (primiparous: 6.88 g/dL, 95% CI 6.60 - 7.15 g/dL; multiparous: 6.89 g/dL, 95% CI 6.68 - 7.10 g/dL) but PCV was relatively lower for calves born from heifers (primiparous: 39.2%, 95% CI 36.7 - 41.8%; multiparous: 42.6%, 95% C 40.6 - 44.6%, P = 0.040). Linear regression (Table 3.6) confirmed that dam parity had no measurable impact (P = 0.109) on PTP concentration at 2 days of age after correcting for colostrum quality and PCV.

Table 3.4: Bulls used to sire study calves, number of calves sired by each bull and mean gestation length (± s.d.) for each bull.

Bull name	**Number of calves**	**mean gestation length (days)**	**Standard deviation**
Bluesky	6	284.8	4.12
Bogart	5	276.8	3.77
Bolivia	2	284.5	3.54
Bolton	2	281.5	3.54
Captain	1	277.0	0.00
Doge	3	280.7	5.69
Gerrard	4	275.3	4.50
Glen	3	272.0	2.65
Groovy	9	271.7	9.81
Iota	10	275.9	5.93
Irresistable	2	281.0	2.83
Lauthority	2	284.5	0.71
Million	6	276.7	2.42
Montague	1	277.0	0.00
Mr Sam	13	273.9	8.43
Norman	4	281.0	5.47
Rollover	2	276.5	4.95
Samuelo	6	279.3	4.63
Specialist	2	272.0	11.3
Steady	5	280.6	2.41
Struik	1	275.0	0.00
Tiergan	1	282.0	0.00
Twist	2	282.0	1.41
Wyman	1	282.0	0.00
Zebra	7	274.0	11.9
Total	**100**	**277.2**	**7.16**

Table 3.5: Multivariable regression model of the association between birth weight and dam parity, gestation length and date of birth, with bull identity included as a random effect.

Outcome variable: Birth weight (kg)			
Variable	**Coefficient**	**95% CI**	**P Value**
dam parity	5.204	4.540 – 5.868	<0.001
gestation length (days)	0.061	0.032 – 0.090	<0.001
tsin4	0.659	0.393 - 0.924	n/a
tsin2	0.506	0.215 – 0.798	n/a
tcos4	0.226	-0.032 – 0.484	n/a
tcos2	-1.371	-1.682 - -1.059	n/a
constant	21.690	13.737 – 29.644	<0.001

Residual ICC=22.4% (95% CI 13.7 - 34.6%)

Random-effects Parameters		
	Estimate (variance)	**95% CI**
Bull Identity	5.186	2.849 - 9.441
Residual error	17.873	16.904 – 18.897

Table 3.6: Association between plasma total protein at 2 days of age with dam parity and colostrum quality after adjusting for Packed Cell Volume.

Plasma total protein	**Coefficient**	**95% CI**	**P Value**
Dam parity (primiparous or multiparous)	-0.274	-0.612-0.063	0.109
Colostrum Quality	0.039	0.004-0.074	0.029
PCV	0.025	0.004-0.046	0.023
Constant	5.165	4.044-6.286	0.000

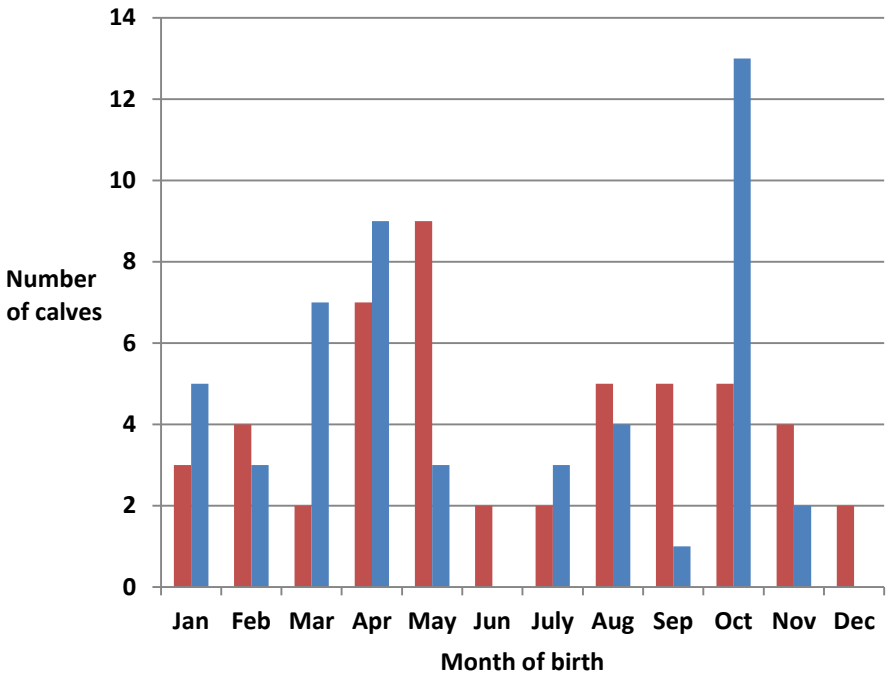

Figure 3.4: Number of calves born in each month of the year. Blue bars indicate calves that were allocated to Group A, red bars indicate calves in Group R.

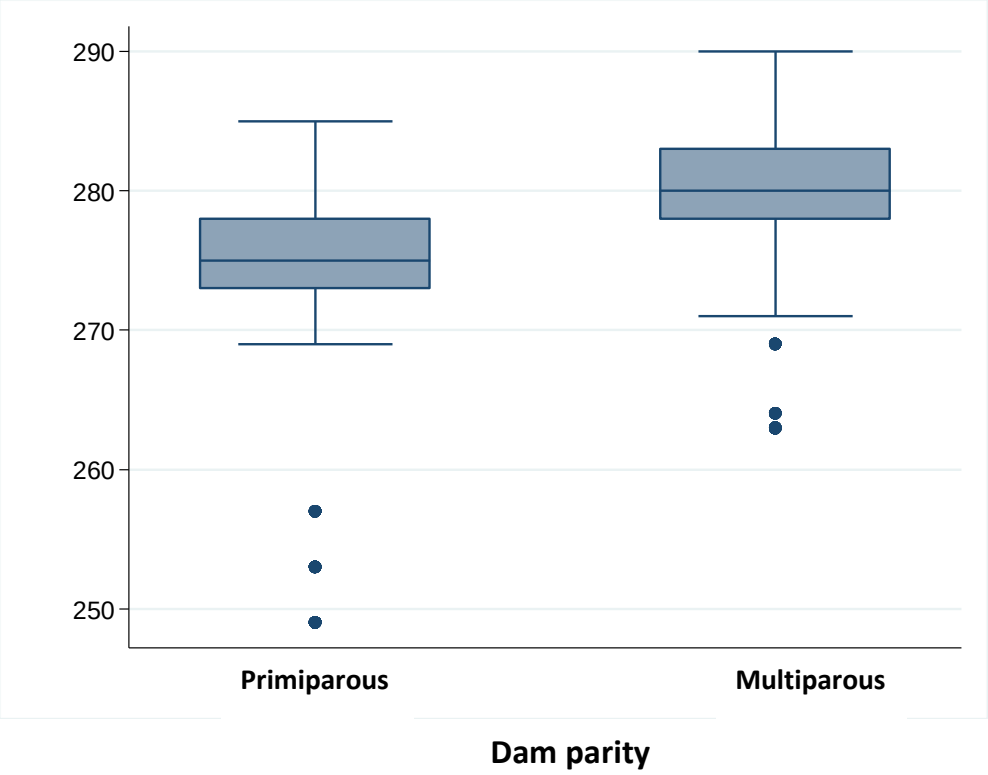

Figure 3.5: Box plot of gestation length for calves born from primiparous (*n* = 43) or multiparous dams (*n* = 57).

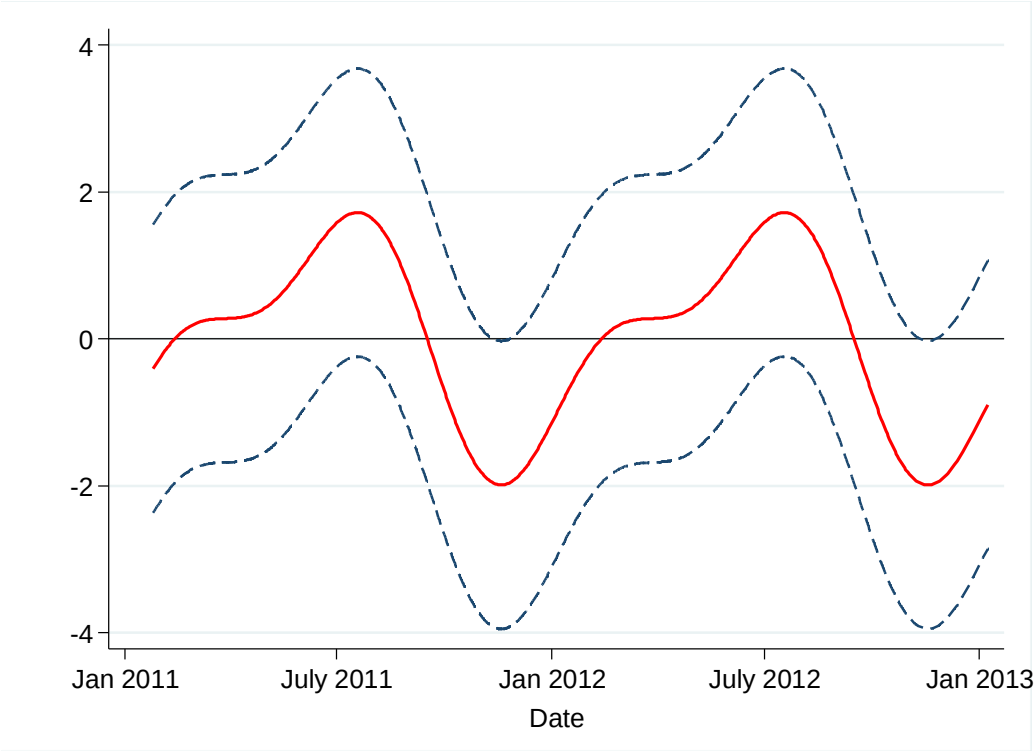

Figure 3.6: Predicted impact of date of birth on the birth weight (95% CI) of calves in the study. The red solid line represents the linear prediction and the blue dotted lines show 95% confidence intervals.

Calves from birth to 12 weeks

Consumption of MR and concentrate feed: All calves consumed all transitional milk offered during the first 4 days of life. All calves in Group R completely consumed MR offered at each meal and total pre-weaning MR consumption was 311 litres. Group A calves consumed considerably more MR (mean 914 litres, 95% CI 873 - 947) than Group R animals over the entire pre weaning period (Figure 3.7a). Voluntary daily MR intakes in Group A calves increased rapidly to reach 7.6 L/day (95% CI 6.5 - 8.7) by day 5. Although not statistically significant overall, MR intakes decreased slightly for Group A animals between days 5 and 9. Thereafter, overall, mean daily MR intake increased linearly to reach 13.3 L (95% CI 12.4 - 14.2) by day 26 before the rate of increase slowed to peak at 15.3 litres per day (95% CI 14.2 - 16.4) near the onset of gradual weaning on day 64. The maximum daily MR intake recorded for any calf was 25.5 litres. Over the 3 week course of MR withdrawal, intakes declined at an overall average rate of 0.7 L daily (Figure 3.2). When data were corrected for body weight and ME, the maximum mean energy provision from MR for calves in Group A was 0.54 MJ/kg body weight/day at 3 weeks of age compared to 0.34 MJ/ kg body weight/day in Group R.

Concentrate feed intakes were negligible (< 50g) for calves in both dietary groups from birth to 3 weeks. After this time, voluntary intakes of concentrate feed for Group R calves gradually increased to approximately 1.0 Kg daily by 7 weeks of age. Conversely, concentrate intakes were relatively less for Group A calves and had only attained < 0.5 Kg/head daily by the onset of weaning at week 8 (Figure 3.7b).

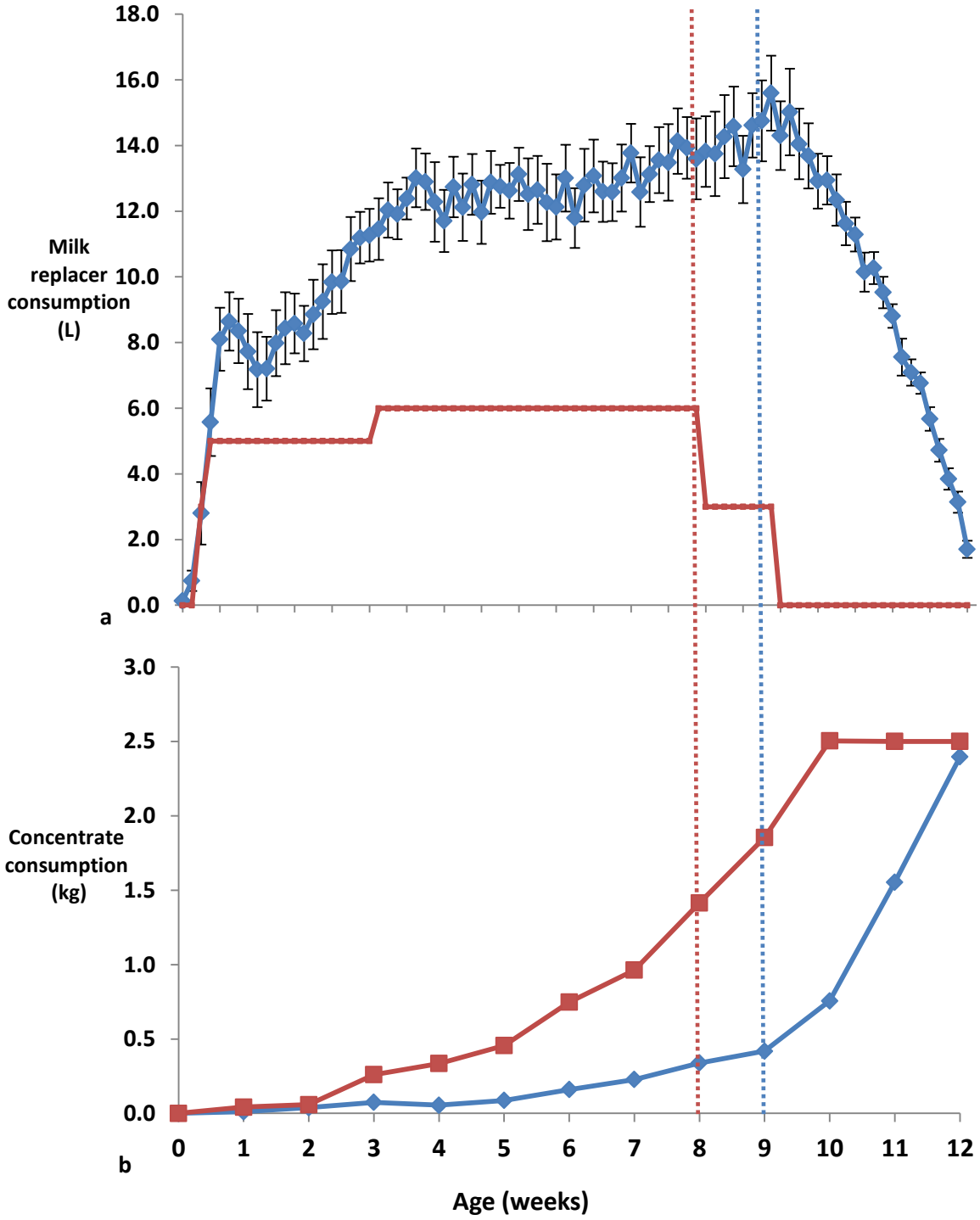

Figure 3.7: a) Mean (95%CI) daily MR consumption for calves in Group A (blue line), and MR allowance for Group R animals (red line) and **b)** mean weekly concentrate intakes (Kg) for a subset of calves in Group R (red line, *n* = 7) and Group A (blue line, *n* = 9) from birth until 12 weeks of age. Dotted lines indicate the onset of weaning for both groups (blue line; Group A, red line; Group R).

Body weight: Pre-weaning nutrition was associated with marked differences in growth rates over time. Overall, average daily gains in body weight (ADG, kg/d) over the first 12 weeks were greater for Group A calves than for Group R animals ($P < 0.001$). When the 12 week pre-weaning period was considered as 4 consecutive 3 week blocks, it was apparent that while Group A calves outperformed Group R animals in the first 9 weeks, Group R animals had advantageous growth rates in the final 3 week period (Table 3.7).

Univariable regression analysis was used to evaluate the unadjusted effects of dietary group, PTP, colostrum quality, disease and dam parity on body weight. All variables, with the exception of PTP and disease were identified as having a significant impact on growth rates (Table 3.8).

From the 2 level random effects multivariable model for body weight, explanatory variables that remained in the model were: dietary group (*ad libitum* or restricted MR) with an interaction with age in weeks, dam parity, the presence of disease, temperature range, minimum temperature, minimum humidity and the four time variables (seasonal effects). Dietary group and calf identity were included as random intercepts and week was included as a random slope (Table 3.9).

A plot of model-derived predicted marginal means (95% CI) for body weight over time indicated that the impact of dietary group was most marked during the first 3 weeks of life when increase in body weight was minimal for Group R animals. Beyond this time, rate of change in body weight were broadly similar between groups (Figure 3.8a). Early constraints on growth therefore resulted in a right shift of the growth curve for Group R animals which was not compensated within the 12 week period evaluated (Fig 3.8a).

Morphometric measures: Univariable analyses of morphometric data suggested that with the exception of belly girth, all other measures (withers and loin height, heart girth, CRL and HFL) had similar dietary group differences in rates of increase over time. By contrast, 'belly' girth was similar for calves in both dietary groups from 3 to 9 weeks but the rate of gain increased during the final 3 weeks for Group R animals (Table 3.10).

Body condition score was significantly greater in Group A by 2 weeks of age, with Group R animals exhibiting a decrease in BCS during this period. This severe (0.4 unit) decrease in

BCS for Group R calves continued until week 4 before a gradual increase began. In contrast Group A calves demonstrated a gradual increase in BCS throughout the first 12 weeks of life (Table 3.10).

For all morphometric measures, final multivariable random effects linear regression models included dietary group as the explanatory variable of prime interest and an interaction term between dietary group and age in weeks. Remaining explanatory variables after backwards stepwise removal (Table 3.11), model coefficients (Table 3.12) and marginal means (Figures 3.8 and 3.9) are presented (full models are available in Appendix B, Table B.1-B.11).

Table 3.7: Mean average daily weight gains for calves in both Group R and Group A at different time periods throughout the first 12 weeks of life. Time periods are split into: birth until 3 weeks of age, 3 weeks until 6 weeks, 6 weeks until 9 weeks and 9 weeks until 12 weeks of age. The overall average daily weight gains for the whole 0-12 week period are also presented.

Age (weeks)	**Mean average daily weight gain (kg) 95% CI**		**P Value**
	Group R (*n* = 50)	**Group A (*n* = 50)**	
0.00-2.99	0.17 (0.08-0.26)	0.72 (0.61-0.82)	<0.001
3.00-5.99	0.74 (0.68-0.81)	0.91 (0.83-0.99)	0.001
6.00-8.99	0.92 (0.86-0.98)	1.04 (0.94-1.13)	0.019
9.00-11.99	1.04 (0.97-1.11)	0.84 (0.74-0.93)	<0.001
Overall 0-12	**0.72 (0.68-0.75)**	**0.87 (0.83-0.91)**	**<0.001**

Table 3.8: Univariable regression analyses to assess variables that may have impacted on body weight. Results of individual analyses are presented together in one table for ease and results are unadjusted for other variables.

Outcome variable: Body weight			
	Coefficient	95% CI	P Value
Dam parity	5.387	3.594 - 7.180	<0.001
Colostrum quality	0.479	0.275 - 0.682	<0.001
Plasma TP	0.684	-0.443 - 1.812	0.234
Birth weight	0.974	0.820 -1.128	<0.001
dietary group (*ad libitum* or restricted MR)	5.945	4.173 - 7.718	<0.001
disease	0.359	-1.877 - 2.594	0.753

Table 3.9: Multivariable regression model including interaction terms and all variables affecting body weight during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: Body weight	Coefficient	95% CI	P value
adlib (*ad libitum* vs. restricted MR)	2.006	-4.897 - 8.909	0.569
week 1	1.443	0.889 - 1.998	<0.001
week 2	1.844	0.744 - 2.944	0.001
week 3	3.946	2.712 - 5.182	<0.001
week 4	9.350	2.406 - 16.294	0.008
week 5	14.213	7.225 - 21.201	<0.001
week 6	19.825	12.785 - 26.866	<0.001
week 7	25.972	18.869 - 33.076	<0.001
week 8	32.348	25.173 - 39.523	<0.001
week 9	39.241	31.986 - 46.495	<0.001
week 10	46.110	38.767 - 53.452	<0.001
week 11	53.110	45.672 - 60.549	<0.001
week 12	61.606	54.064 - 69.147	<0.001
adlib#week 1	2.045	1.269 - 2.821	<0.001
adlib#week 2	3.635	2.092 - 5.178	<0.001
adlib#week 3	9.218	7.490 - 10.945	<0.001
adlib#week 4	9.203	2.121 - 16.285	0.011
adlib#week 5	10.426	3.260 - 17.591	0.004
adlib#week 6	11.769	4.502 - 19.036	0.002
adlib#week 7	12.446	5.062 - 19.831	0.001
adlib#week 8	13.261	5.744 - 20.779	0.001
adlib#week 9	14.160	6.496 - 21.824	<0.001
adlib#week 10	12.561	4.735 - 20.387	0.002
adlib#week 11	12.909	4.909 - 20.909	0.002
adlib#week 12	9.907	1.720 - 18.093	0.018
dam parity	6.132	4.196 - 8.069	<0.001
disease	-3.471	-5.929 - -1.013	0.006
age at 1st colostrum	-0.357	-0.793 - 0.079	0.109
temperature range	-0.083	-0.158 - -0.008	0.031
humidity range	-0.021	-0.045 - 0.002	0.074
min. temperature	0.131	0.061-0.201	<0.001
min. humidity	-0.033	-0.054 - -0.011	0.003
tsin4	-2.641	-3.277 - -2.005	n/a
tsin2	1.681	0.526 - 2.836	n/a
tcos4	0.417	-0.272 - 1.107	n/a
tcos2	-0.392	-1.661 - 0.877	n/a
constant	43.872	36.675-51.069	<0.001

Random-effects Parameters (variance)	Estimate	95% CI
group:	10.235	3.194 - 32.794
calf:	27.344	20.222 - 36.974
week	0.845	0.635 - 1.125
Residual	9.854	9.270 - 10.475

Table 3.10: Mean average daily morphometric gains for calves in Group A and Group R throughout the first 12 weeks of life. Time periods are split into 1) birth until 3 weeks of age, 2) 3 weeks until 6 weeks, 3) 6 weeks until 9 weeks and 4) 9 weeks until 12 weeks of age. The overall average daily weight gains for the whole 0-12 week period are also presented.

Age (weeks)	**Mean average daily withers height gain (cm)**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**0.18**	**0.25**	**<0.001**
3.00-5.99	**0.21**	**0.26**	**<0.001**
6.00-8.99	**0.23**	**0.26**	**0.090**
9.00-11.99	**0.20**	**0.26**	**0.002**
Overall 0-12	**0.21**	**0.26**	**<0.001**
Age (weeks)	**Mean average daily loin height gain (cm)**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**0.15**	**0.22**	**0.001**
3.00-5.99	**0.23**	**0.30**	**<0.001**
6.00-8.99	**0.22**	**0.22**	**0.427**
9.00-11.99	**0.18**	**0.21**	**0.070**
Overall 0-12	**0.19**	**0.24**	**<0.001**
Age (weeks)	**Mean average daily Heart girth gain (cm)**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**0.20**	**0.39**	**<0.001**
3.00-5.99	**0.37**	**0.45**	**0.005**
6.00-8.99	**0.37**	**0.42**	**0.045**
9.00-11.99	**0.35**	**0.32**	**0.155**
Overall 0-12	**0.32**	**0.40**	**<0.001**
Age (weeks)	**Mean average daily Belly girth gain (cm)**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**0.26**	**0.60**	**<0.001**
3.00-5.99	**0.66**	**0.58**	**0.103**
6.00-8.99	**0.68**	**0.65**	**0.344**
9.00-11.99	**0.65**	**0.44**	**0.003**
Overall 0-12	**0.56**	**0.57**	**0.357**
Age (weeks)	**Mean average daily crown-rump length gain (cm)**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**0.29**	**0.33**	**0.217**
3.00-5.99	**0.28**	**0.52**	**<0.001**
6.00-8.99	**0.36**	**0.35**	**0.399**
9.00-11.99	**0.35**	**0.38**	**0.265**
Overall 0-12	**0.32**	**0.40**	**<0.001**
Age (weeks)	**Mean average daily hock-fetlock length gain (cm)**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**0.08**	**0.09**	**0.331**
3.00-5.99	**0.05**	**0.09**	**<0.001**
6.00-8.99	**0.06**	**0.07**	**0.314**
9.00-11.99	**0.07**	**0.06**	**0.414**
Overall 0-12	**0.06**	**0.08**	**<0.001**
Age (weeks)	**Mean average Body Condition Score change**		
	Group R (*n* = 50)	**Group A (*n* = 50)**	**P value**
0.00-2.99	**-0.311**	**0.103**	**<0.001**
3.00-5.99	**0.104**	**0.009**	**0.007**
6.00-8.99	**0.043**	**0.009**	**<0.001**
9.00-11.99	**0.004**	**-0.008**	**<0.001**
Overall 0-12	**0.120**	**0.316**	**0.018**

Table 3.11: Explanatory variables included in the final regression models investigating factors associated with morphometric measure changes from birth to 12 weeks of age.

Outcome Variable	Explanatory Variables	Random effects Variables	Interactions
Withers height	Dietary group dam parity volume of first colostrum feed temperature range illness time variables	calf group calf identity	dietary group X age(weeks)
Loin height	Dietary group dam parity volume of first colostrum feed temperature range age at first colostrum feed illness time variables		
Heart girth	Dietary group dam parity illness plasma total protein time variables		
Belly girth	Dietary group dam parity illness volume of first colostrum feed temperature range plasma total protein time variables		
Crown to rump length	Dietary group dam parity illness temperature range volume of first colostrum feed time variables		
Hock-fetlock length	Dietary group dam parity volume of first colostrum feed minimum temperature temperature range time variables		
Body condition score	Dietary group dam parity plasma total protein minimum temperature humidity range time variables		

Table 3.12: Coefficients (95% CI) for all remaining explanatory variables in the 7 morphometric multivariable models. Coefficients for dietary group and interaction terms are not presented.

Outcome variable / **Explanatory variables**	withers height	loin height	heart girth	belly girth	crown to rump length	hock-fetlock length	body condition score
plasma total protein			0.485 (-0.071 - 1.042)	0.589 (-0.201 - 1.380)			0.039 (0.006 - 0.072)
dam parity	1.461 (0.581 – 2.342)	1.351 (0.429 - 2.273)	3.551 (2.619 - 4.483)	5.109 (3.758 - 6.460)	2.007 (0.648 - 3.365)	0.486 (0.142 – 0.829)	0.049 (-0.007 -0.104)
presence of disease	-0.800 (-1.862 – 0.261)	-0.749 (-1.863 - 0.365)	-1.655 (-2.823 - -0.488)	-3.093 (-4.789 - -1.380)	-1.097 (-2.778 - 0.583)		
volume of 1st colostrum feed	0.762 (0.008 – 1.516)	1.003 (0.208 - 1.797)		1.185 (0.010 - 2.360)	1.125 (-0/056 - 2.307)	0.545 (0.245 – 0.845)	
age at first colostrum feed		-0.217 (-0.413 - -0.020)					
min. temperature						-0.057 (-0.091 - -0.024)	-0.007 (-0.013 - -0.001)
min. humidity							
temperature range	0.049 (0.013 - 0.085)	0.036 (-0.010 - 0.083)		0.138 (0.026 – 0.249)	-0.084 (-0.186 - 0.018)	-0.038 (-0.068 - -0.008)	
humidity range							0.002 (0.001 - 0.003)
tsin4	-0.054 (-0.272 - 0.164)	0.059 (-0.188 - 0.306)	0.214 (-0.069 - 0.498)	0.263 (-0.296 – 0.823)	0.607 (0.107 - 1.106)	-0.112 (-0.256 – 0.032)	0.024 (-0.002 - 0.049)
tcos4	-0.133 (-0.363 - 0.098)	-0.151 (-0.411 - 0.109)	0.184 (-0.112 - 0.481)	-0.400 (-1.000 – 0.200)	0.097 (-0.447 - 0.640)	0.477 (0.324 – 0.630)	-0.066 (-0.092 - -0.041)
tsin2	0.431 (0.078 – 0.784)	0.155 (-0.256 - 0.566)	-0.010 (-0.448 - 0.428)	0.193 (-0.596 – 0.983)	-0.112 (-0.890 - 0.665)	-0.285 (-0.490 - -0.080)	-0.041 (-0.075 - -0.006)
tcos2	-0.298 (-0.680 - 0.084)	-0.143 (-0.581 - -0.294)	-0.383 (-0.843 - 0.076)	-0.903 (-1.772 - -0.035)	1.313 (0.376 - 2.251)	-0.267 (-0.530 - -0.004)	0.018 (-0.023 - 0.059)

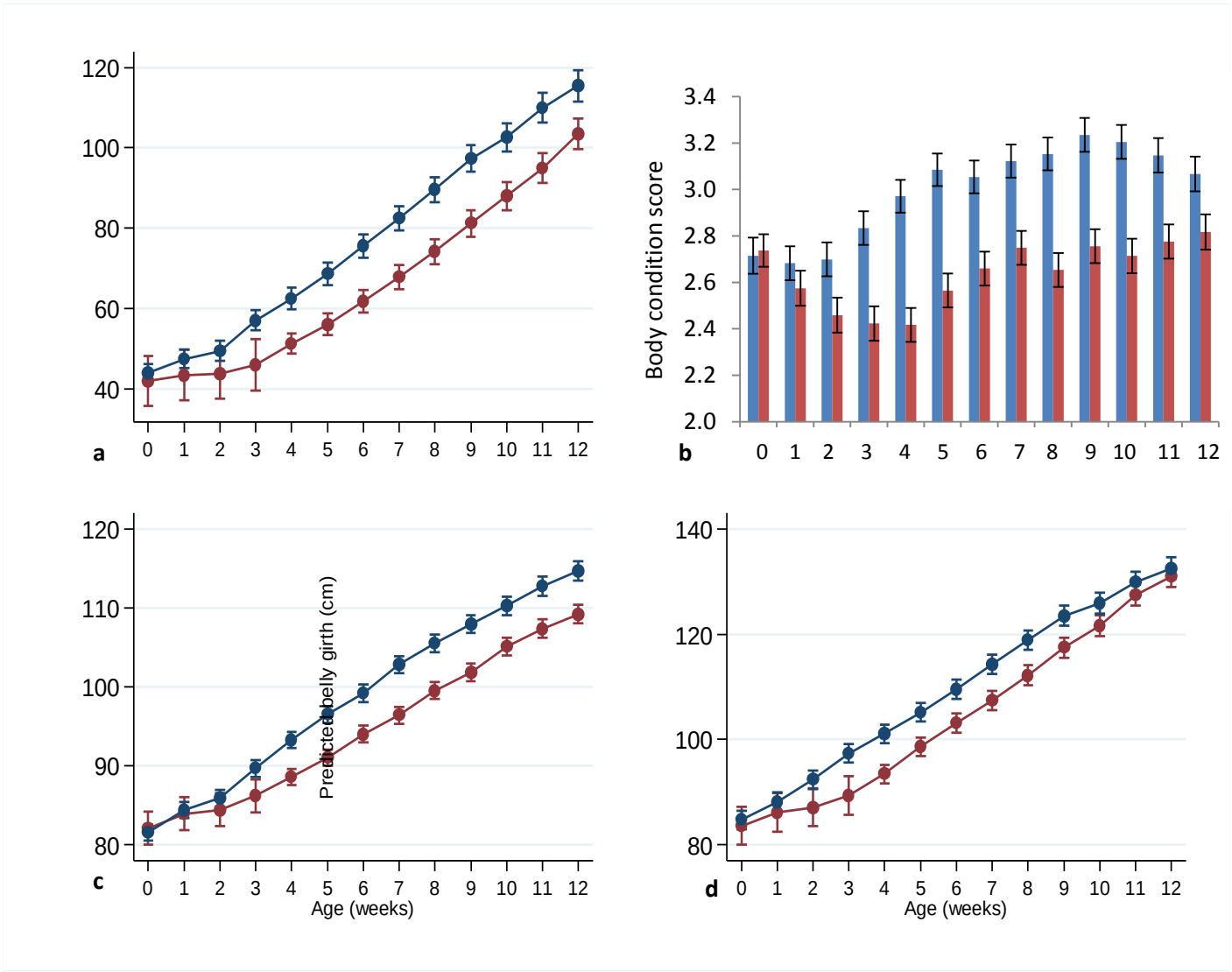

Figure 3.8: Marginal means (95% CI) of a) predicted body weight (kg), b) predicted BCS, c) predicted heart girth (cm) and d) predicted belly girth (cm) for calves in Group A (blue line) and R (red line) from birth until 12 weeks of age.

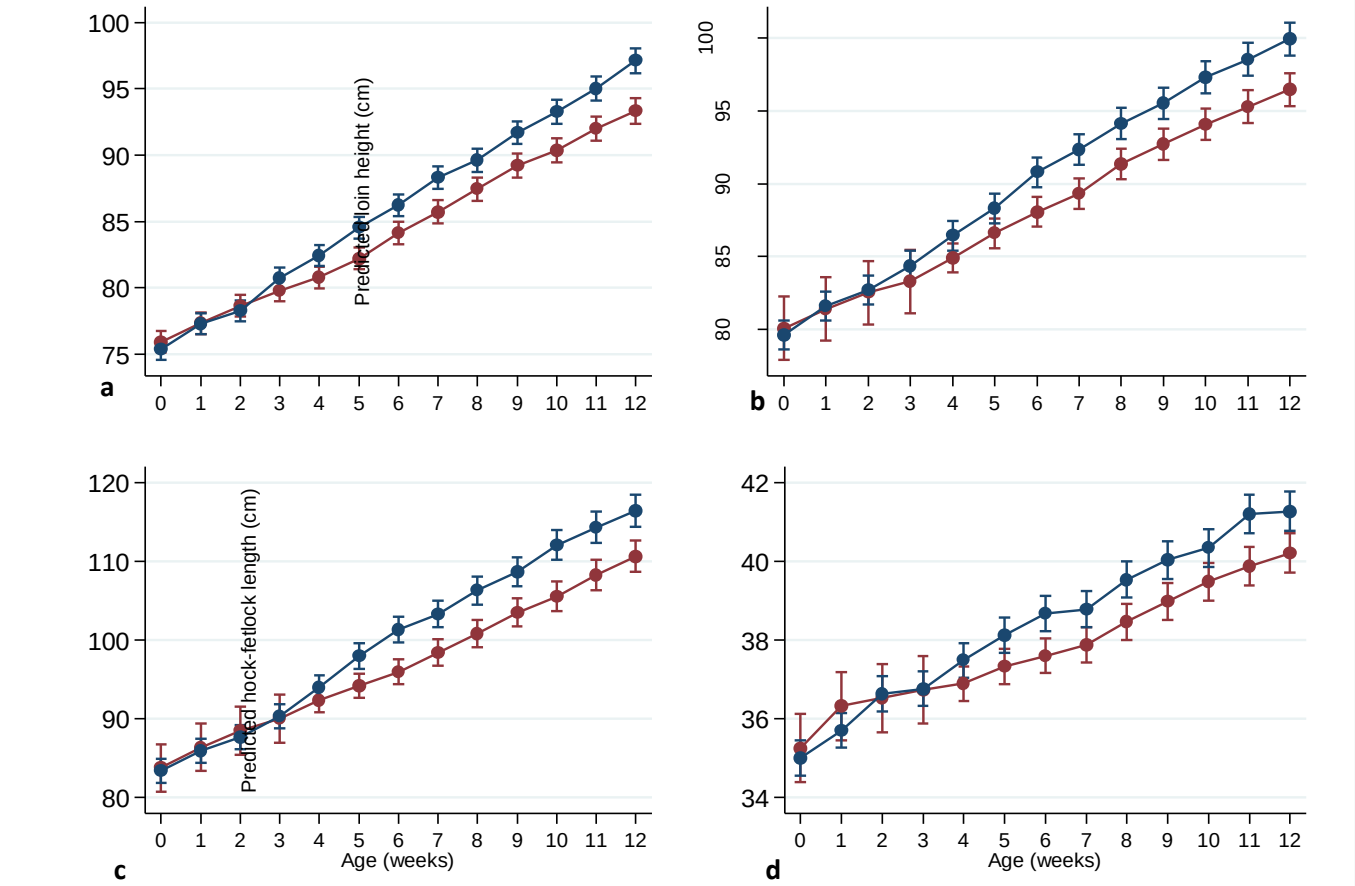

Figure 3.9: Marginal means (95% CI) of a) predicted withers height (cm), b) predicted loin height (cm), c) predicted crown rump length (cm) and d) predicted hock-fetlock length (cm) for calves in Group A (blue line) and R (red line) from birth until 12 weeks of age.

Disease: All calves presenting with clinical signs of disease were treated appropriately, the mortality rate was 0%. Faecal samples obtained from 3 calves presenting with neonatal diarrhoea identified rotavirus as the causative agent of disease.

In total, 80 (80%) calves suffered from at least one incident of disease during the period from birth to 12 weeks. Calves in Group R had a lower incidence of disease (*n* = 33, 66%, 95%CI 52 - 80%) than Group A (*n* = 47, 94%, 95% CI 87 - 100%, P < 0.001).

In total there were 57 cases of diarrhoea affecting 56 calves (one calf in Group A had two separate incidents). Of the primary incidents, 36 occurred in Group A and 20 in Group R. There were 42 cases of pneumonia affecting 37 calves (5 calves suffered a second incidence of disease). Of the primary incidents, 28 occurred Group A and 9 in Group R. Four calves in Group A and one in Group R suffered a second case.

Group A calves had a significantly higher risk of exhibiting symptoms of diarrhoea or pneumonia, with unadjusted odds ratios of 3.86 (95% CI 1.67 - 8.91) for diarrhoea and 5.80 (95% CI 2.33 - 14.44) for pneumonia. Univariable analysis showed no effect of dam parity, colostrum quality, PTP or birth weight on the likelihood of an episode of either diarrhoea or pneumonia (Table 3.13). Mean age of onset of a case of diarrhoea was 9.9 days (95% CI 8.2 - 11.6 days, range 1 - 28 days) and for pneumonia was 48.2 days (95% CI 42.2 - 54.0 days, range 28- 77 days, Figure 3.10). Mean age of diagnosis of pneumonia was significantly higher in the Group A calves compared to Group R (52.1 days *versus* 36 days P = 0.016) (Figure 3.12.)

There was considerable variation in the incidence of both diarrhoea and pneumonia throughout the study. No pneumonia cases were recorded in the first 5 months of the study, and thereafter there was only one case until the winter of 2012, when more cases were recorded. Diarrhoea was seen throughout the majority of the study period with only the final 2 months being free from diarrhoea (Figure 3.11).

The minimum and maximum temperatures and humidity's of the calf house were recorded daily (Figure 3.13). For prolonged periods during January and February of each year of the study, the ambient temperature within the calf house was below the stated lower critical

temperature (10°C) for a calf of up to 3 weeks of age. There were no consistent periods of time where the upper critical temperature (25^0C) of young calves was reached in this study.

The relative humidity within the calf house fluctuated greatly throughout the course of the study (21 - 99%). Relative humidity was above the optimum range for a young calf throughout the study period with minor exceptions.

Multivariable analysis demonstrated that there was no association between dam parity, birth weight, and colostrum quality and the likelihood of occurrence of a case of diarrhoea or pneumonia, leaving dietary group (*ad libitum* or restricted MR) ($P < 0.05$) and in the case of diarrhoea, PTP ($P = 0.183$) as the remaining explanatory variables in the models (Table 3.14).

Table 3.13: Odds ratios derived from univariable logistic regression models; including explanatory variables associated with the probability of an episode of either diarrhoea or pneumonia.

Outcome variable: Episode of Diarrhoea			
Explanatory variable	**Odds Ratio**	**95% CI**	**P Value**
Ad libitum MR	3.86	1.67 - 8.91	< 0.001
Dam parity	1.167	0.542 - 2.510	0.693
Colostrum quality	0.994	0.912 - 1.083	0.888
Plasma TP	1.195	0.734 - 1.948	0.474
Birth weight	0.958	0.896 - 1.024	0.210
Outcome variable: Episode of Pneumonia			
Explanatory variable	**Odds Ratio**	**95% CI**	**P Value**
Ad libitum MR	5.80	2.33 – 14.44	< 0.001
Dam parity	1.289	0.581 - 2.859	0.532
Colostrum quality	1.023	0.935 - 1.119	0.619
Plasma TP	1.037	0.629 - 1.711	0.887
Birth weight	0.992	0.927 - 1.061	0.806

Table 3.14: Random effects multivariable logistic regression model for both diarrhoea and pneumonia, including explanatory variables associated with the probability of a disease episode.

Outcome variable / Explanatory variable	**Diarrhoea**		**Pneumonia**	
	Odds ratio (95% CI)	P Value	Odds ratio (95% CI)	P Value
ad libitum MR	3.986 (1.684 - 9.437)	0.002	5.798 (2.328 - 14.438)	<0.001
Plasma TP	1.441 (0.842 - 2.467)	0.183		
constant	0.052 (0.001 - 2.308)	0.127	0.220 (0.107 - 0.452)	<0.001

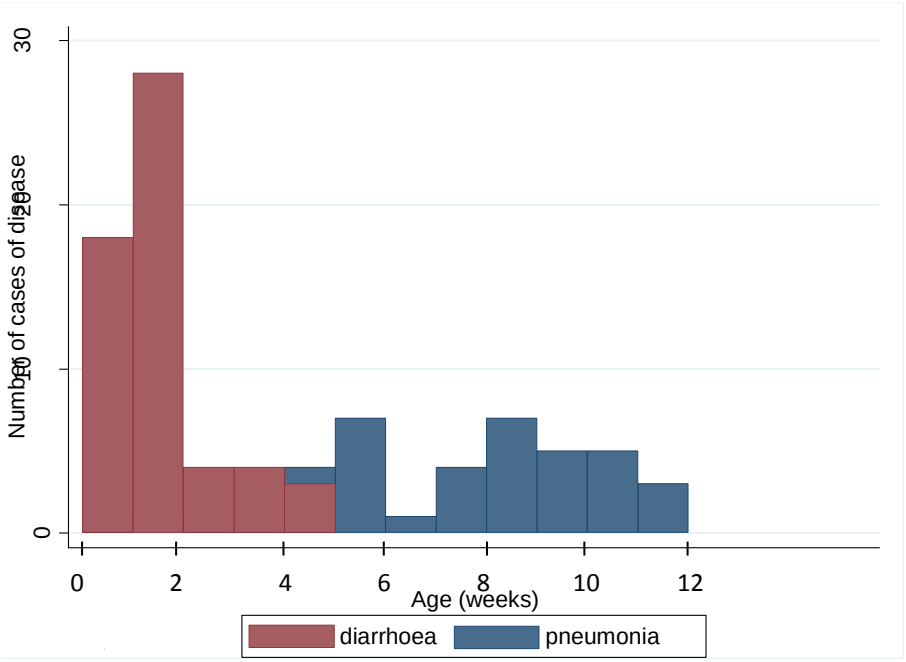

Figure 3.10: Occurrence of diarrhoea (red bars) and pneumonia (blue bars) by age of calf from birth to 12 weeks of age.

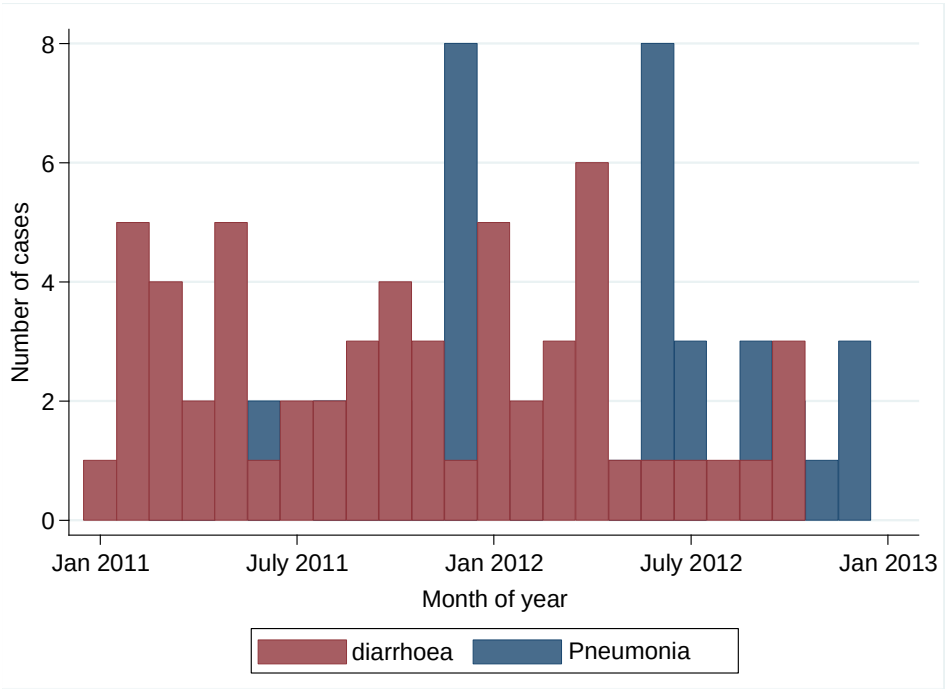

Figure 3.11: Total number of cases of diarrhoea (red bars) and pneumonia (blue bars) throughout the study (January 2011 - January 2013).

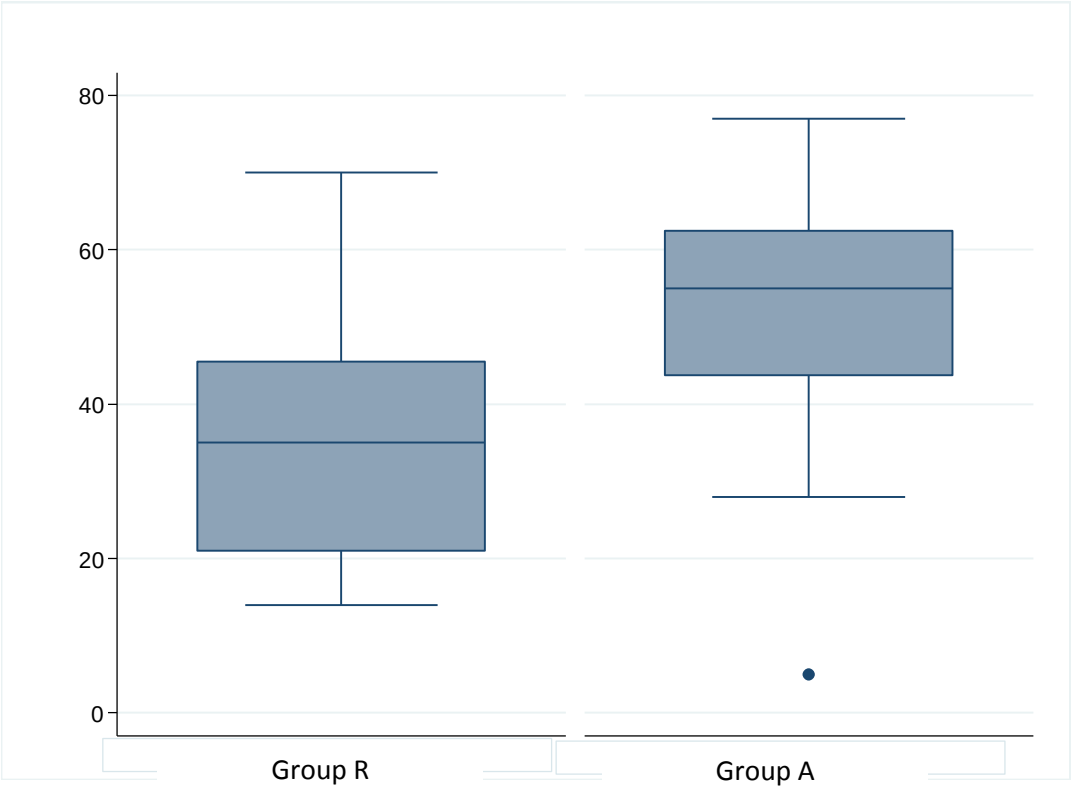

Figure 3.12: Box plot of age (in days) of age at pneumonia diagnosis for Group R and Group A calves.

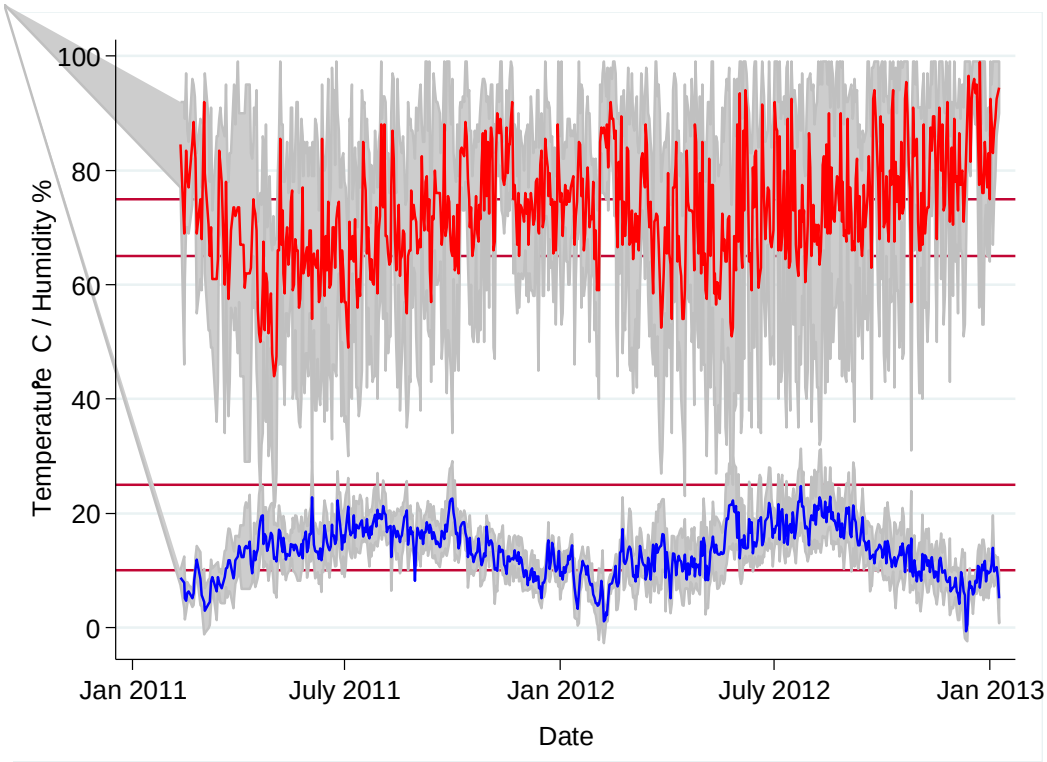

Figure 3.13: Mean temperature (blue line) and humidity (red line) in the calf house for the duration of the study. Minimum and maximum temperatures and humidities are illustrated by the grey shaded areas; reference lines at 10°C and 25°C and 65% and 75% depict optimum environmental temperature and humidity ranges respectively for young calves.

3.4 Discussion

Dairy calf rearing strategies have traditionally been based on least cost principles, often feeding milk or milk replacer to only 10% of body weight daily (Andreia De Paula et al., 2008; Hepola, 2003; Jasper and Weary, 2002; Kehoe et al., 2007). Recognition of the impact of this on the health, welfare and missed opportunity for optimal growth has driven research on the topic of increased milk feeding for young calves (de Passille et al., 2011; Hill et al., 2013; Moallem et al., 2010; Richard et al., 1988; von Keyserlingk et al., 2006).

In this study, the body weight of calves born from a cow in her second lactation or above was greater than that of calves born from primiparous dams. This supports much of the literature available (Dhakal et al., 2013; Swali and Wathes, 2007) reporting that as heifers have not reached mature body weight at service (approximately 60%) (Lynch et al., 1997) they therefore give birth to smaller calves. The parity of the dam was not taken into account when calves were assigned to dietary group during this study, which may have been a limitation. However, dam parity was included as a potential confounder in all analyses thereby allowing estimation of its effects, if any.

From the multivariable regression model, after accounting for dam parity, bull and gestation length, the predicted birth weight of calves was 2kg less during the winter than the summer. Although the effect of season on birth weights of calves has been studied previously, there have been contradictory findings. Birth weights of calves have been higher in those born in spring than in autumn (Odde et al., 1985). Heat stress in summer months during gestation has been found to cause a reduced birth weight of calves in the autumn (Bonsma, 1949). Long term exposure of dams to high environmental temperatures causes a reduction in birth weight of calves, likely due to elevated blood temperatures. However, the above reasons are unlikely to affect the birth weight of calves in the current study as chronic exposure to high temperatures did not occur.

Dam nutrition may impact on the birth weight of calves. A study carried out in Hereford cows found that reducing the plane of dam nutrition in the third trimester, caused a decrease in calf birth weight by 6.8kg (Tudor, 1972). Similarly manipulation of the diet during early to mid gestation influenced the birth weight of calves from beef heifer dams

(Micke et al., 2010). In contrast, other work has suggested that the birth weight of a calf is not influenced by the diet of the dam unless there is significant long term nutritional deficiency (Holland and Odde, 1992).

In the present study, all pre-partum animals were housed and fed to specifically cater for their stage of production. There was likely to be little if any seasonal variation in diet offered, suggesting that this was not the causal mechanism underlying these differences in birth weight.

The colostrum quality, as measured by specific gravity, of primiparous dams did not differ from colostrum of multiparous cows in this study in contrast to some previous studies. Historically, heifers have been reported to produce colostrum of reduced quality (Morin et al., 1997; Shearer et al., 1992). The current findings are in agreement with more recent studies suggesting that colostrum from primiparous dams is not inferior to colostrum from multiparous dams in terms of quality (Godden, 2008; Gulliksen et al., 2008).

Calves with *ad libitum* access to MR consumed significantly higher volumes than calves in the restricted MR fed group. At peak volumes, the mean daily MR intake was approximately 16 litres compared to the 6 litre allowance of the restricted fed group. It was therefore not surprising that the corresponding body weights of calves in the *ad libitum* MR fed group were significantly higher than the restricted fed calves during the study period. The largest difference in body weight was seen in the early period from birth until 3 weeks of age, with Group A animals achieving growth rates of 0.72kg per day compared to Group R calves gaining only 0.17kg per day.

Calves in Group R only started to show significant increases in body weight from 4 weeks of age onwards, whereas Group A calves gained weight from birth, with growth rates remaining approximately the same throughout the 12 week period. The advantages in body weight gained during the early period in the *ad libitum* fed group were maintained throughout the first 12 weeks of life. Even though growth rates of Group R calves increased to over 1kg daily during the 9 to 12 week period, these calves were unable to carry out sufficient 'catch-up growth' to match the body weight of Group A animals by the end of the 12 week period.

As newborn calves are mono-gastric with undeveloped rumens, concentrate feed provided from birth to 3 weeks of life was not consumed during this period in either group. (Drackley, 2008). It is apparent that the limited volume of MR fed twice daily in the Group R was completely insufficient to support growth during the first few weeks of life.

The apparent nutritional inadequacy of restricted MR provision in Group R is demonstrated by the decrease in BCS during the first 4 weeks. During this period, calves were likely to have been metabolising body tissues for maintenance and growth. In stark contrast to this, BCS increased consistently from birth to 9 weeks for calves belonging to Group A.

On reaching 4 to 5 weeks of age, concentrate intake increased rapidly in Group R, enabling rumen development and increase in function, and providing accessibility of nutrients for growth.

Group R calves were highly motivated to consume additional concentrate feed at an earlier age due to the limited amount of MR provided, unlike their *ad libitum* fed counterparts. Calves in Group R who were fed 2.5 litres of MR twice daily from birth to 3 weeks were maintained in a state of 'chronic hunger' during this time. Supporting evidence for this statement includes behavioural studies where calves fed restricted volumes of MR via a computerised feeder, compared to calves offered *ad libitum* quantities spent more time visiting the feeder and competed more with other calves in an attempt to find more milk (De Paula Vieira et al., 2008). This would suggest that the restricted fed calves were dissatisfied with the volumes of milk replacer offered and were therefore highly motivated to drink or eat more.

The present study raises major welfare concerns regarding the volume of milk or milk replacer fed to calves during the first 3 weeks of life, bearing in mind that many farmers feed less than 2.5 litres twice daily at this stage of life (Chapter 2).

Most U.K. dairy farmers state that they aim for approximate growth rates throughout the pre-weaned period of 0.7kg daily using restricted MR systems. From earlier work carried out within this thesis (Chapter 2), less than 1% of dairy farmers within a study population of 723 regularly weighed their young calves and growing heifers. The findings from the current chapter would suggest that most U.K. dairy farmers are not able to accurately monitor

growth rates in young calves and that many calves on farms in the U.K. are in fact growing at much slower rates than predicted. The disparity in growth rates between the 2 groups of calves observed in this study would reinforce the recommendation that monitoring of growth of young calves should be carried out on a regular basis.

Changes over time of other morphometric measures of growth (withers and loin height, heart girth, crown-rump length and hock-fetlock length), corresponded broadly to changes in body weight measures and were significantly higher for Group A than Group R throughout the study. The exception to this was belly girth, primarily a measure of "gut fill", which was greater in Group A until 9 weeks of age. After 9 weeks, differences in belly girth measures between the 2 groups began to reduce and by 12 weeks of age there were no dietary group differences.

Overall belly girth measures did not differ between the 2 dietary groups from birth to 12 weeks although the intermediate time points throughout the 12 week period showed Group R calves having a lower mean belly girth measure. The difference between the 2 groups was most apparent during the first 3 weeks of life, most likely a measure of 'gut fill', with Group R calves unable to consume sufficient concentrate feed to give a rounded appearance to the belly. The difference in belly girth measures between groups disappeared as calves approached 12 weeks of age. This is likely due to the onset of weaning at 9 weeks of age in Group A, and the ability for Group R animals to now consume sufficient forage and concentrate feed to give a rounded appearance. However, all other morphometric measures were smaller for Group R calves at 12 weeks of age, demonstrating that belly girth measures were not in proportion with the rest of their body. The concentrate intake for calves in both dietary groups was restricted to a maximum of 2.5kg daily. Whilst Group A calves consumed relatively small amounts of concentrates until the start of the weaning period at 9 weeks, Group R calves consumed all their concentrate feed from an earlier age with intakes starting to increase from 3 weeks of age onwards (Figure 3.7). The calves in Group R were also consuming significant amounts of forage, and although forage intake was not measured, this may explain why these animals had a 'pot bellied' appearance and corresponding belly girth measure.

Interestingly, although the body weight and BCS of calves in Group A was significantly higher than that of the Group R from 2 weeks of age, the corresponding differences in all other morphometric measures were not statistically significant until between 3 and 6 weeks of age. There appeared to be a "lag period" between body weight and BCS whereby dietary group differences in these measures were apparent in the first three weeks of life but differences in other morphometric measures did not appear until the calves were 3-4 weeks old. Increased MR intake in Group A enabled calves to utilise increased energy intakes and optimise growth during this important stage of life. The delay in the differences in skeletal growth may be for a number of reasons. The first being that during the first few weeks of life, immunological challenge of the naive immune system of a calf is very energy demanding, therefore increased growth rates as measured by skeletal growth may be delayed until this process has been completed. Another explanation may be that although Group R calves had a significantly lower body weight by 2 weeks of age compared to Group A calves. The calves in Group R utilised body tissues and fat reserves to ensure skeletal growth was in line with Group A fed animals until approximately 4 weeks of life. This second hypothesis is further supported by data from the body condition scores of these calves.

Disease of young dairy calves has a large economic impact on the industry as a whole (Roy and Ternouth, 1972), with morbidity and mortality of young calves being most commonly caused by diarrhoea and pneumonia (Gorden and Plummer, 2010; Windeyer et al., 2014). Failure of passive transfer of immunoglobulins exposes calves to a higher risk of morbidity and mortality during early life; intensity of disease is reduced in calves with a serum Ig concentration of at least 10 g/L (Furman-Fratczak et al., 2011). However, failure of passive transfer is not the sole factor determining disease, there are usually multiple factors affecting disease risk such as management strategies, hygiene, temperature and humidity (Lorenz et al., 2011c; McGuirk, 2007; Roy and Ternouth, 1972; Smith, 2003).

Disease incidence in this study was high, with 80% of animals exhibiting at least one disease episode. As no calves suffered from failure of passive transfer of immunoglobulins (as measured by plasma TP concentrations at 48 hours of age), other factors must have been associated with the high disease incidence observed. Disease was categorised as either diarrhoea or pneumonia based on presenting symptoms; no other disease conditions were observed in the cohort. Neonatal diarrhoea was recorded when faeces were of a looser

consistency than normal calves for >1 day. Pneumonia was recorded when calves had at least one of the following symptoms: coughing, nasal or ocular discharge or increased respiratory sounds and elevated rectal temperature (> 39.5^0 C). There were 57 cases of diarrhoea in total with significantly more of the Group A animals affected. Diarrhoea was seen most frequently during the first 2 weeks of life, with some cases during weeks 3 and 4; this is a typical pattern of neonatal calf diarrhoea incidence (García et al., 2000). There are many causative agents of neonatal diarrhoea, the most common are *E. coli, Salmonella sp.,* Cryptosporidium, Rotavirus and Coronavirus (Lorenz et al., 2011b). Many of these organisms are ordinarily present in a farm environment (Garber et al., 1994; Izzo et al., 2011) but the naive immune system of a young calf means that these animals are more susceptible to disease. In the present study, rotavirus was identified in diarrhoeic faecal samples suggesting it was the causal agent.

There were 42 cases of pneumonia recorded in this study, again with a higher incidence in Group A compared to Group R calves (32 vs 26 cases). Usually, pneumonia is seen in calves from approximately 6 weeks of age. Data from this study is in agreement with this. Respiratory disease can be caused by a multiplicity of organisms, both viral and bacterial including Respiratory Syncitial Virus (RSV), Parainfluenza 3 (PI3), *Mycoplasma spp*. and *Pasteurella spp* (Lorenz et al., 2011a).

The marked age difference in susceptibility to clinical pneumonia between dietary groups (54 days for Group A *versus* 35 days for Group R) initially appeared counter-intuitive since pathogen challenge was probably greater for Group A animals due to the teat feeder. It may therefore be expected that these calves would succumb to disease at an earlier age than restricted MR fed animals. The association between immune response and plane of nutrition in the young calf is poorly understood with contradictory findings depending on which aspect of the immune response is scrutinised (Ballou, 2012; Ballou et al., 2015; Obeidat et al., 2013). However Ballou *et al* (2015) found an enhanced immune response to challenge with an oral *Salmonella enterica* var *typhimurium* vaccine in calves fed increased levels of MR during the pre-weaning period. This suggests that increased nutrition may be associated with an enhanced immune response. We may hypothesise that the dietary group age difference observed in the present study was associated with an increased resilience to pathogen challenge in *ad libitum* MR fed animals.

Disease in neonatal calves is known to cause increased mortality, reduced growth rates and increased age at first calving (Wathes et al., 2008). However in the present study, the mortality rate was 0% and the growth rate of diseased animals during the first 12 weeks of life was not apparently reduced. This may have been due to rapid identification and treatment of disease in this cohort, associated with the large amounts of time spent by the researcher with these animals on a daily basis.

Group housing of calves may have a significant impact on disease transmission between individuals within a group (Gorden and Plummer, 2010). Furthermore, the use of automatic teat feeders may exacerbate problems with direct contact transfer of disease causing pathogens (Hepola, 2003; Maatje et al., 1993) via the communal use of one teat. The increased incidence of disease seen in Group A calves of this study is likely due to a combination of these factors. Calves assigned to Group R were individually housed until approximately 21 days of age, reducing the risk of disease transmission during the period in which neonatal scours may have a large impact on health of these animals unlike Group A calves who were group housed from birth facilitating transmission of pathogens associated with neonatal diarrhoea.

Regardless of dietary group, calves were housed in the same building and therefore shared air-space. Group pens had solid side partitions to reduce direct pathogen transmission between groups. Thus all calves were exposed to the same environmental risk factors for pneumonia. As could be seen from the recorded environmental data (temperature and humidity), the housed environment was severely sub-optimal and likely contributed to the overall high disease rates observed. The increased incidence of pneumonia observed in Group A calves was likely to be associated with the use of a single teat for multiple calves. The teat is an excellent transmission vehicle for respiratory pathogens via saliva and nasal secretions. Another factor in this increased incidence may be that Group A calves will have produced significantly more urine and liquid faeces due to increased consumption of water. This increased the likelihood of wet bedding and its associated risks. Furthermore calves in Group A were still feeding from the automatic teat feeder up until 12 weeks of age, further exposing them to risk of respiratory disease transmission during this time. This increased respiratory disease incidence in *ad libitum* MR fed calves has previously been reported (Hepola, 2003).

There is considerable anecdotal evidence regarding the association between the use of automatic milk feeding machines and increased risk of pneumonia. This has usually been explained in terms of large group size (> 20 animals) and mixing of age groups (Hepola, 2003). Neither of these factors were relevant in the present study thus clearly illustrating the increased risk associated with machine feeding out with group size and age considerations.

The results of the current study suggest that modifications are required to automatic feeding machines to reduce the impact of this transmission route e.g. by including teat disinfection procedures between calves visiting the feeding station. Furthermore, the present study would suggest that such automatic feeding systems should only be employed in housing facilities that provide a low background risk for calf pneumonia.

Data from a farmer questionnaire study in Sweden found that farmers using a computerised milk feeder for calves took more time caring for them. They were then able to identify health disturbances more frequently and more rapidly (Beckman, 1993). This is certainly true of the present study; calves were handled at least once and checked 3 times daily, enabling early detection of any disease in both groups of calves.

Surprisingly, no clear seasonal fluctuations in disease incidence were evident in the present study, although there did appear to be more cases of diarrhoea in the late winter and spring months. The thermo-neutral zone of a calf under 21 days of age is between 10 and 25°C, above or below which extra energy is expended to carry out thermoregulation (Gonzalez-Jiminez and Blaxter, 1962; Schrama et al., 1993). The environmental conditions in the rearing facility in the present study were relatively poor both in terms of temperature and humidity, often being too cold and too humid (Figure 3.12). In order to combat this, calves less than 21 days of age were fitted with jackets to reduce energy requirements for thermoregulation.

In conclusion, the *ad libitum* milk replacer feeding of neonatal calves resulted in higher body weight and in turn, greater skeletal growth than calves fed restricted volumes of MR throughout the first 12 weeks of life. The lifetime benefits associated with increased MR feeding are predicted to outweigh the costs of feed purchase by reducing time to puberty, first service, conception and therefore entry into the milking herd. In addition to an earlier

age to productivity, increased milk yield during the first and subsequent lactations will further increase the profitability of these heifers as milking adults. There have been various studies assessing the impact of increased milk or MR feeding on productivity during the first lactation, with increases in milk yield of between 450 and 1400kg (Bar-Peled et al., 1997; Drackley et al., 2007; Soberon et al., 2012). The corresponding improvement to animal welfare by fulfilling the nutritional needs of these animals as neonates is also an important consideration for dairy calf producers. The increased incidence of disease in Group A calves in this study was assumed to be an effect of shared teat usage in group housed animals. Further work is required to specifically evaluate this, with a view to improving the design of computerised teat feeders so that calf health is not compromised.

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Chapter 4

Effect of milk replacer feeding strategy on the carcass composition of Holstein bull calves

4.1 Introduction

Approximately half a million Holstein bull calves are born in the U.K. annually (Beyond Calf Export Forum, 2013). These animals were historically classed as a by-product of the industry and deemed almost worthless by the market place. Nearly 85,000 dairy bull calves were disposed of on farm at birth in 2006 and 81,000 were exported live for fattening in other countries where there was greater demand for this type of meat (Beyond Calf Export Forum, 2013). The welfare implications associated with live export of young animals into countries with poorly regulated welfare standards are considerable and remain a key concern within the U.K. dairy sector.

Over the past 8 years, consortia of academics, producers, processors, retailers and non-government organisations have worked together to develop dedicated supply chains for these dairy bull calves within the U.K.. Successful collaborations have effected a 90% reduction in live exports, decreased the numbers of calves killed on farm by 36% and increased the number of animals retained in the U.K. for incorporation into the beef supply chain by 58% (Beyond Calf Export Forum, 2013). Various strategies to increase markets for the use of dairy bull beef and to a lesser extent veal, have been implemented (Ashfield et al., 2014). The use of sexed semen to reduce the birth of unwanted bull calves has made a small impact on the number of unwanted bull calves produced. However, the long term aim should be to breed robust dairy animals with a suitable carcass conformation for meat production.

The dairy industry generally rears calves on least cost principles, feeding limited amounts of milk or milk replacer (MR) to ensure a swift transition onto solid feed stuffs (Thomas et al., 2001). These restricted milk feeding practices are increasingly considered to be a welfare issue with calves often being maintained in a state of chronic hunger during the early stages of life (Andreia De Paula et al., 2008). Not only does this have a negative impact on welfare, it also limits opportunity for growth during the period in which the highest feed conversion rate may be achieved.

In terms of growth of heifer calves for future performance in the milking herd, the key aim should be to ensure sufficient growth to allow entry into the milking herd by 24 months of age (Haworth et al., 2008; Keown and Everett, 1986). Growth targets for the dairy beef

industry are similar. The primary objective for dairy beef is to achieve a marketable carcass weight by 18 – 24 months of age.

The provision of restricted milk or MR for dairy calves is still commonplace on U.K. dairy farms and has previously been justified by anecdotal or short term evidence (Anderson, 2011). Studies assessing rumen characteristics of veal calves fed large volumes of milk have reported minimal rumen development (Baldwin et al., 2004; Heinrichs and Lesmeister, 2005). Early transition from MR to cheaper solid feedstuffs has been considered an important economic objective for dairy producers. However, early transition to solid feeds often deprives the calf of sufficient dietary energy to support growth in early life. This may outweigh the short term financial gains by constraining long term production potential.

Veal calves are often fed *ad libitum* milk in conjunction with low levels of concentrate and forage feeds to ensure sufficient weight gain prior to finishing (Webb et al., 2012). Although *ad libitum* milk fed animals have decreased voluntary forage and concentrate intakes over the pre weaning period than conventionally fed animals (Jensen and Budde, 2006; Quigley et al., 2006), this was not associated with reduced post weaning intakes (Borderas et al., 2009a; Borderas et al., 2009b; de Passille et al., 2011; Jasper and Weary, 2002). Drackley *et al* (Drackley et al., 2007) demonstrated that calves fed *ad libitum* MR and weaned at 6 weeks of age, gained a weight advantage over calves fed MR in restricted amounts. Furthermore, this study clearly indicated that *ad libitum* MR fed calves produced significantly larger volumes of milk than restricted MR fed animals during lactation 1 (Drackley et al., 2007). It is likely that a similar production advantage could be achieved for dairy beef by the adoption of *ad libitum* or increased MR feeding.

Increased adipose deposition in dairy calves has also been offered as an argument against increased milk or MR feeding programs. There has been some evidence to suggest that calves fed increased energy and protein prior to weaning store more adipose tissue than calves fed a standard protein and energy diet (Brown et al., 2005). Obesity poses a major threat to health and fertility in dairy cows, and it is especially important that animals do not enter into lactation with excess body fat (Bisinotto et al., 2011; Douglas et al., 2006; Grant and Keown, 1993; Sinclair, 2010). It is equally important to avoid excess adipose tissue deposits in bull calves reared for beef.

Optimal development of mammary parenchymal tissues is vital for potential high yielding dairy cattle, without which, the genetic capability of milk production will never be realised and economic losses to the dairy producer may occur (Daniels, 2010). Historically, negative effects on mammary development were believed to be associated with increased milk or MR feeding and this was attributed to increased growth rates during early life (Lammers et al., 1999; Sejrsen, 1994). However, more recent and rigorous studies have found no effect of increased early growth rates on mammary development in dairy heifers (Daniels et al., 2009).

Further work is required to evaluate any differences in the core physical components of dairy bull calves fed different milk replacer allowances in the early phase of life.

The primary objective of this study was to compare the carcass composition of dairy bull calves, at 3, 9 and 12 weeks of age, with access to either restricted or *ad libitum* MR. We tested the hypothesis, that providing calves with *ad libitum* access to MR would not have significantly higher proportions of carcass fat compared to calves with restricted access at any age. A secondary study objective was to compare rumen weights of calves in both dietary groups.

4.2 Materials and Methods

The study was conducted in compliance with the University of Liverpool Veterinary Ethics Committee and the U.K. Animals (Scientific Procedures) Act, 1986.

Animals and husbandry

Twenty-one Holstein bull calves were recruited between May and August 2011 from calves born within the University of Liverpool's, Wood Park Dairy Farm, Neston, Wirral, U.K. and maintained under the husbandry conditions described below until euthanasia at pre-determined ages. Calves were born into group accommodation with between 5 and 15 cows present and remained with their dam for up to 18 hours post-calving.
Sequentially born bull calves were assigned to dairy heifer rearing-groups ($n \leq 6$ per group), such that the ages of individual animals in each group ranged by no more than 14 days. Alternate rearing groups were pre-designated to receive one of two milk replacer (MR) feeding strategies: Group A; *ad libitum* MR access ($n = 9$) or Group R; restricted MR access ($n = 9$, Table 4.1). A further 3 calves were recruited, euthanased and studied within 2 hours of birth.

Between 3 and 4 litres of calves own dam's colostrum (collected as soon as possible after birth) was administered to each calf via an oesophageal feeder, and given to the calf at the earliest opportunity after birth, together with further, freshly-collected, dam-specific colostrum meals (fed via individual bucket) twice daily (2 litres per feed) for four days before beginning MR feeding (96.97% DM, 22.17% crude protein, 19.76% oil, 7.02% ash, ME 21.570 MJ/kgDM, pH 5.96, Blossom Easy Mix, Volac, U.K.). For calves in Group A, familiarisation and training for use of the automatic computerised teat feeder (Vario feeder, Forster Technik, Germany) from which *ad libitum* MR was dispensed began from birth. Calves in this group were able to access MR from birth in addition to the 4 day dam specific colostrum meals.

The specific gravity of the initial colostrum meal was assessed using the sample collected at birth with a Brix refractometer (Animal Reproduction Systems, CA, U.S.A.). Blood samples (20ml) were collected by jugular venipuncture into plain and heparinised 10ml vacutainers

at 48 hours after birth (Beckton Dickinson & Son Ltd, Oxford, U.K.). Plasma total protein concentration (PTP) was estimated by refractometry (Clinical refractometer, Hayes, U.K.).

Immediately prior to feeding, powdered MR was thoroughly mixed with water (125g MR/litre, 37^{O}C). Group R calves were offered MR from day 4, predefined as 5 litres daily until 3 weeks of age, then 6 litres daily thereafter until weaning onset at 8 weeks. Calves were offered MR twice daily, equally divided into two meals fed at 09:00 and 17:00 hrs (Table 4.1). The age and timing of weaning from MR differed between the 2 dietary groups. Group R began weaning at 56 days of age (8 weeks) by reduction of MR provision to 3 litres once daily, fed at 09.00 hrs for 1 week until complete cessation of MR feeding at 63 days (9 weeks). Group A began weaning at 9 weeks with restriction to 16 litres daily, progressive further restriction followed by mean reduction by 0.75 litres daily until completion of weaning at 84 days (12 weeks) of age (Figure 4.1). Concentrate feedstuffs (Primestart coarse mix, 86.2% DM, 18% crude protein, 8% crude fibre, 9.5% ash, 3.5% oil, ME 14.459MJ/Kg, BOCM Pauls Ltd U.K.) were available to all calves (to a maximum intake of 2.5 kg per head) throughout the 12 weeks study. All calves had *ad libitum* access to forage (grass hay and wheat straw bedding) and water throughout the study period.

Calves in group R were housed individually in metal gated pens (1m x 2m) over raised slatted flooring and bedded with wheat straw from birth until 21 days of age. At 21 days of age, Group R calves were moved to deep wheat straw-bedded group pens (5m x 6m) ($n \leq 6$, age range ≤ 14 days). Calves in Group A were grouped by age (range ≤ 14 days, $n \leq 6$) and were directly introduced to group pens on entry into the calf house. All calves had *ad libitum* access to forage (grass hay), fresh water and coarse mix concentrate feed, up to a maximum of 2.5 kg per head daily.

Table 4.1: Nutritional and husbandry protocols used for all calves in the Group A (*ad libitum* MR access) and Group R (restricted MR access).

Group	Milk Replacer allowance	Milk Replacer feeding method	Weaning Protocol	Housing Method	Concentrate and Forage
A	*Ad libitum* access until day 63	Automatic teat feeder	Stepwise restriction of daily MR allowance over 21 days	Group housed from birth (n ≤ 6)	*Ad libitum* access to grass hay and 2.5kg concentrate feed (coarse mix) daily
R	5L daily until day 21, then 6L daily until day 56 (provided as 2 equal meals, (09:00 & 17:00hrs)	Individual bucket to day 21, thereafter group trough fed	50% reduction of MR allowance over 7 days	Individually housed until 21 days then group housed (n ≤ 6)	*Ad libitum* access to grass hay and 2.5kg concentrate feed (coarse mix) daily

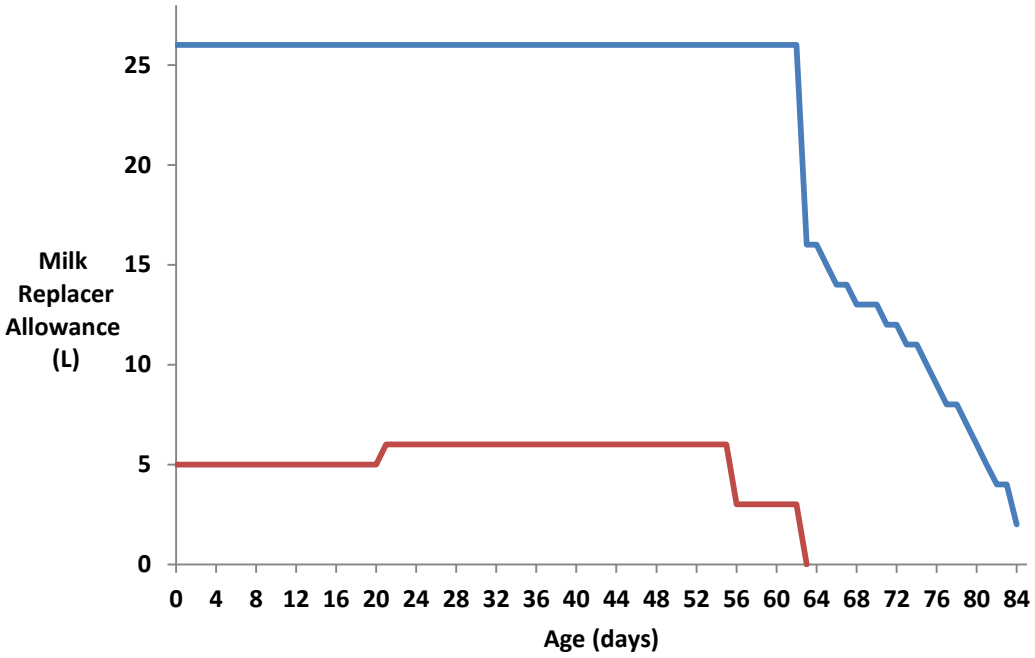

Figure 4.1: Milk replacer allowance (litres/day) for calves in Group R (red line, *n* = 9) and Group A (blue line, *n* = 9) from birth to weaning.

Experimental Procedure

Calves were removed from their rearing groups at birth (*n* = 3), 3 weeks (Group A: *n* = 3, Group R: *n* = 3), 9 weeks (Group A: *n* = 3, Group R: *n* = 3) or 12 weeks (Group A: *n* = 3, Group R: *n* = 3). Measures of height at the highest point of the withers (±0.1cm, wooden measuring stick, I&D Smallwood, U.K.) and body condition score (BCS) was recorded in accordance with the system presented by Edmonson *et al* (Edmonson et al., 1989).

The body weight of each calf was recorded (Ritchey Ltd, North Yorkshire, U.K., ±0.5kg) ten minutes prior to euthanasia (by captive bolt [*n* = 16] or barbiturate overdose [*n*= 5]). Exsanguination was carried out immediately *post-mortem* and blood lost at exsanguination was collected and weighed (±10g, Weigh-Tronix, West Midlands, U.K.).

Dissection of all carcasses followed a prescribed order. The head was removed at the atlanto-occipital joint and the carcass hide (including the tail) was removed and weighed (±10g). Carcasses (including the limbs and hooves) were eviscerated using a ventral midline incision. The complete gastro-intestinal tract (GIT) was ligated (300mm x 4.8 mm plastic cable ties) to secure region-specific digesta (reticulo-rumen, omasum, abomasum, small intestine, caecum and colon). The tract was subsequently divided and 'full' and 'empty' (following digesta evacuation, rinsing with water and blotting to dry) weights of each region were used to calculate the corresponding digesta mass (± 1g, Salter, Kent. U.K.).

All viscera were individually weighed and organ-associated adipose tissues were removed and weighed separately (±0.1g, Appendix D, Table D.1-D.5).

The remaining empty carcass was sagittally sectioned and the spinal cord was removed and weighed (±0.1g, Appendix D). Left and right carcass sides were weighed (±0.5 kg) and the right half carcass was sealed in plastic and stored at -20°C prior to virtual dissection by Spiral CT-Analysis.

Spiral CT-Analysis

Half carcasses were removed from -20°C storage and thawed to 4°C during transport before spiral CT scans were performed at the SRUC-BioSS CT Scanning unit, Edinburgh, U.K. Spiral scans produced a series of cross sectional images at 8mm intervals (Siemens Somatom Esprit CT scanner, Munich, Germany, Figure 4.2). Image analysis was performed using STAR software to determine total carcass tissue (fat, lean and bone) volumes and densities. Total tissue weights were calculated from all cross sectional images by compounding tissue volume by tissue density (weight = volume * density). Values generated from the CT image data of right side carcasses were multiplied by 2 to gain whole carcass composition. CT image data were expressed as a percentage of empty body weight, where empty body weight was the total body weight less digesta.

Data from the CT analysis and weights from dissection were combined to allow calculation of *ante-mortem* and *post-mortem* weight differences.

Differences between *ante-mortem* and final total recovered weights were assumed to be water loss from evaporation during the dissection and the freeze/thaw process.

Statistical Analyses

Calves at Birth: Simple univariable analysis using Students t tests was initially carried out to investigate associations between variables. Variables of interest were birth weight, colostrum quality and plasma TP concentration at 48 hours.

Calves prior to euthanasia: Mann-Whitney U tests were used to assess dietary group differences in body weight, withers height and body condition score.

Carcass composition: Mann-Whitney U tests were used to assess differences in proportions of bone, skeletal muscle, skeletal adipose tissue, abdominal adipose tissue, hide, viscera, rumen-reticulum, gastro-intestinal tract (not including rumen-reticulum) and fluid as a percentage of empty body mass for calves in both dietary groups at 3, 9 and 12 weeks of age.

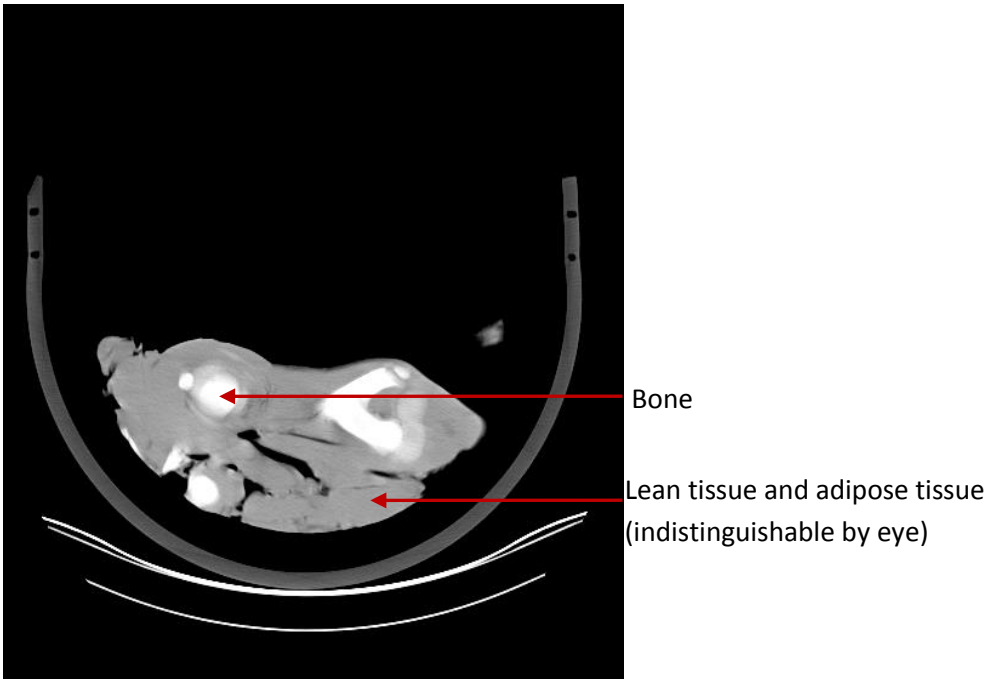

Figure 4.2: Image of one ‘slice’ from a spiral CT of one calf carcass.

4.3 Results

Dam parity was not associated with calf birth weight (mean 47.6 kg, 95% CI 45.2 - 49.9, P = 0.060, *n* = 21), specific gravity of *peri-partum* colostrum (mean 22.1%, 95% CI 20.0 - 24.3, P = 0.200, *n* = 18) or 48 hour PTP concentrations (mean 6.77 g/dL, 95% CI 6.35 - 7.19, P = 0.470, *n* = 18).

The median body weight and withers height of calves euthanased at 3 weeks differed between dietary group (Group A; 68.5 kg, 84.5cm, Group R; 49.5 kg, 77.4cm P = 0.050), but this difference was not apparent for calves euthanased at 9 weeks (100.8 kg, P = 0.827; 93.5 cm, P = 0.383) or 12 weeks (125.0 kg, P = 0.513; 97.3 cm, P = 0.827).

There were no dietary group differences in BCS at any age (3 weeks; BCS = 2.7, P = 0.487. 9 weeks; BCS = 3.3, P = 0.275. 12 weeks; BCS = 3.3, P = 0.197). All weight, height and BCS measures for bull calves in this study were within ranges recorded for heifer calves of the same age (Table 4.2).

For all calves at all ages, there were no visually apparent sub-cutaneous adipose tissue deposits. For calves studied at 3 weeks of age, there were no dietary group differences in bone, skeletal muscle, skeletal adipose tissue, hide, fluid, gastro-intestinal tract (not including rumen-reticulum) and viscera (all organs, urogenital tract, testicles, brain and spinal cord) as a percentage of empty body mass. However Group A calves had significantly greater abdominal adipose tissue (P = 0.050) and significantly lower rumen-reticulum weights as a percentage of empty body mass than Group R calves (P = 0.050, Table 4.3, Figures 4.3 and 4.4).

At 9 weeks of age, calves from Group A had a greater skeletal muscle (P = 0.050), lower skeletal adipose tissue (P = 0.050) and lower rumen-reticulum weights (P = 0.050) as a percentage of empty body mass than Group R animals (Table 4.3, Figures 4.3 and 4.4).

At 12 weeks, Group A calves had a greater abdominal adipose tissue (P = 0.050), greater hide (P = 0.050) and lower rumen-reticulum weight (P = 0.050) as a percentage of empty body mass than Group R calves (Table 4.3, Figures 4.3 and 4.4).

There was no association between BCS and carcass associated adipose tissue over all age ranges (P = 0.196).

At 3, 9 and 12 weeks of age Group R calves had a greater rumen-reticulum weight as a percentage of empty body mass, compared to *ad libitum* MR fed calves. However, on visual inspection there were no gross differences between dietary groups (Figure 4.5).

Table 4.2: Body weight (kg), withers height (cm) and body condition score (BCS) (Edmonson et al., 1989) of bull calves prior to euthanasia at: birth (*n* = 3, new born), 3 (*n* = 6), 9 (*n* = 6) and 12 weeks (*n* = 6) of age in Group A and Group R. The ranges for heifer calves (*n* = 100; 50 Group A, 50 Group R) from the larger intervention study (Chapter 3) at corresponding ages are presented for comparison.

Group &Age (weeks)	**Body weight (kg)**	
	Bulls (median, *n* = 3/group)	**Heifers (range, *n* = 50/group)**
new born 0	46.5	27.0 - 56.0
R 3	49.5	34.0 - 61.0
A 3	68.5	38.5 - 76.5
R 9	100.0	56.5 - 101.5
A 9	101.5	79.5 - 128.0
R 12	124.5	69.5 - 127.0
A 12	136.0	93.0 - 143.5
	Withers height (cm)	
Group &Age (weeks)	**Bulls**	**Heifers (range)**
new born 0	81.0	67 - 81.8
R 3	77.4	73.5 - 86.0
A 3	84.5	73.2 - 88.5
R 9	93.0	81.2 - 95.8
A 9	94.0	82.6 - 98.0
R 12	97.0	83.5 - 98.0
A 12	97.5	89.5 - 106.0
	BCS	
Group &Age (weeks)	**Bulls**	**Heifers (range)**
new born 0	2.1	2.0 - 3.4
R 3	2.7	1.7 - 3.1
A 3	2.7	1.9 - 3.3
R 9	3.2	2.1 - 3.3
A 9	3.4	2.7 - 3.6
R 12	3.3	2.2 - 3.2
A 12	3.3	2.6 - 3.4

Table 4.3: Median proportion of bone, skeletal muscle, skeletal adipose tissue, abdominal adipose tissue, hide, viscera (all organs, brain, spinal cord, urogenital tract), rumen-reticulum, remainder of the gastro-intestinal tract (gastro-intestinal tract - rumen-reticulum), and fluid recovered from a combination of dissection and CT-analysis of bull calves at birth (*n* = 3) and in Group A and Group R at 3, 9 and 12 weeks of age (*n* = 3 per group).

Type	age (weeks)	median proportion of empty body mass % (range)		P value
		Group A	Group R	
Bone	0	20.34 (16.37 - 20.91)		
	3	17.27 (13.98 - 18.76)	19.48 (18.29 - 19.96)	0.127
	9	16.28)15.46 - 16.39)	12.71 (12.46 - 18.19)	0.513
	12	13.69 (11.96 - 15.77)	15.94 (14.89 - 16.52)	0.127
Skeletal muscle	0	42.22 (41.28 - 42.46)		
	3	40.24 (39.11 - 45.26)	40.30 (38.37 - 40.82)	0.827
	9	41.81 (41.72 - 44.75)	39.17 (36.90 - 39.78)	0.050
	12	38.81 (35.68 - 39.24)	38.24 (37.74 - 39.55)	0.827
Carcass associated adipose tissue	0	9.23 (8.46 - 12.18)		
	3	10.62 (10.38 - 11.67)	9.77 (8.88 - 10.50)	0.127
	9	9.64 (9.49 - 9.83)	12.28 (10.40 - 13.65)	0.050
	12	13.15 (11.98 - 19.50)	11.23 (9.46 - 13.10)	0.127
Internal adipose tissue	0	1.01 (0.63 - 1.28)		
	3	1.14 (0.96 - 1.60)	0.61 (0.41 - 0.78)	0.050
	9	1.72 (1.48 - 1.73)	0.67 (0.60 - 1.11)	0.050
	12	1.68 (1.44 - 2.19)	1.11 (1.04 - 1.35)	0.050
Hide	0	7.64 (7.53 - 9.11)		
	3	7.12 (5.79 - 7.62)	6.72 (6.26 - 6.85)	0.513
	9	7.07 (5.95 - 8.18)	7.96 (7.27 - 8.09)	0.513
	12	7.85 (7.67 - 7.93)	6.38 (6.07 - 7.53)	0.050
Viscera	0	7.48 (7.35 - 7.55)		
	3	7.53 (5.96 - 10.19)	8.32 (7.28 - 8.38)	0.827
	9	6.95 (6.71 - 8.43)	8.26 (7.82 - 9.88)	0.275
	12	7.15 (7.10 - 7.60)	7.97 (7.08 - 13.40)	0.513
Rumen-reticulum	0	0.46 (0.43 - 0.82)		
	3	0.56 (0.52 - 0.71)	0.86 (0.82 - 1.12)	0.050
	9	1.05 (1.02 - 1.49)	3.55 (1.86 - 3.67)	0.050
	12	1.67 (1.60 - 1.96)	2.69 (2.55 - 3.02)	0.050
Remainder of gastro-intestinal tract	0	3.91 (3.66 - 4.13)		
	3	5.03 (4.40 - 5.27)	4.74 (4.39 - 4.85)	0.275
	9	4.40 (4.34 - 6.38)	5.67 (5.25 - 6.11)	0.513
	12	4.80 (4.71 - 5.75)	6.01 (5.73 - 6.91)	0.127
Fluid	0	5.68 (3.92 - 6.08)		
	3	7.60 (5.90 - 11.11)	6.44 (5.54 - 12.61)	0.827
	9	6.51 (5.99 - 8.77)	6.46 (6.44 - 6.50)	0.513
	12	6.95 (6.32 - 7.00)	6.37 (6.23 - 6.58)	0.275

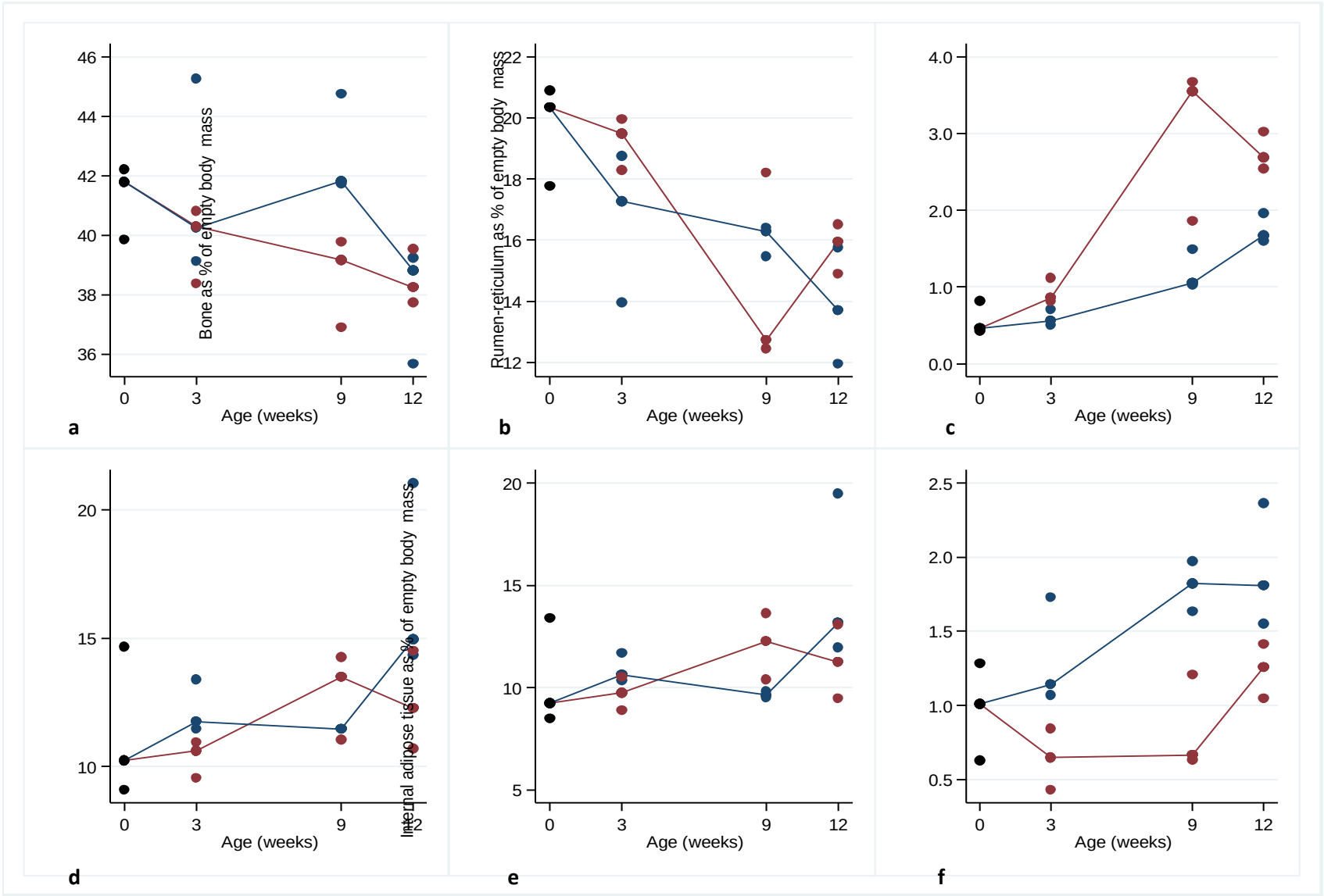

Figure 4.3a) skeletal muscle, **b)** bone, **c)** rumen-reticulum, **d)** total adipose tissue, **e)** carcass adipose tissue and **f)** Internal adipose tissue as a percentage of total empty body mass for individual calves at birth (black), and in Group A (blue) and Group R (red) at 3, 9 and 12 weeks of age. Blue and red lines indicate median values for Group A and R calves at each age.

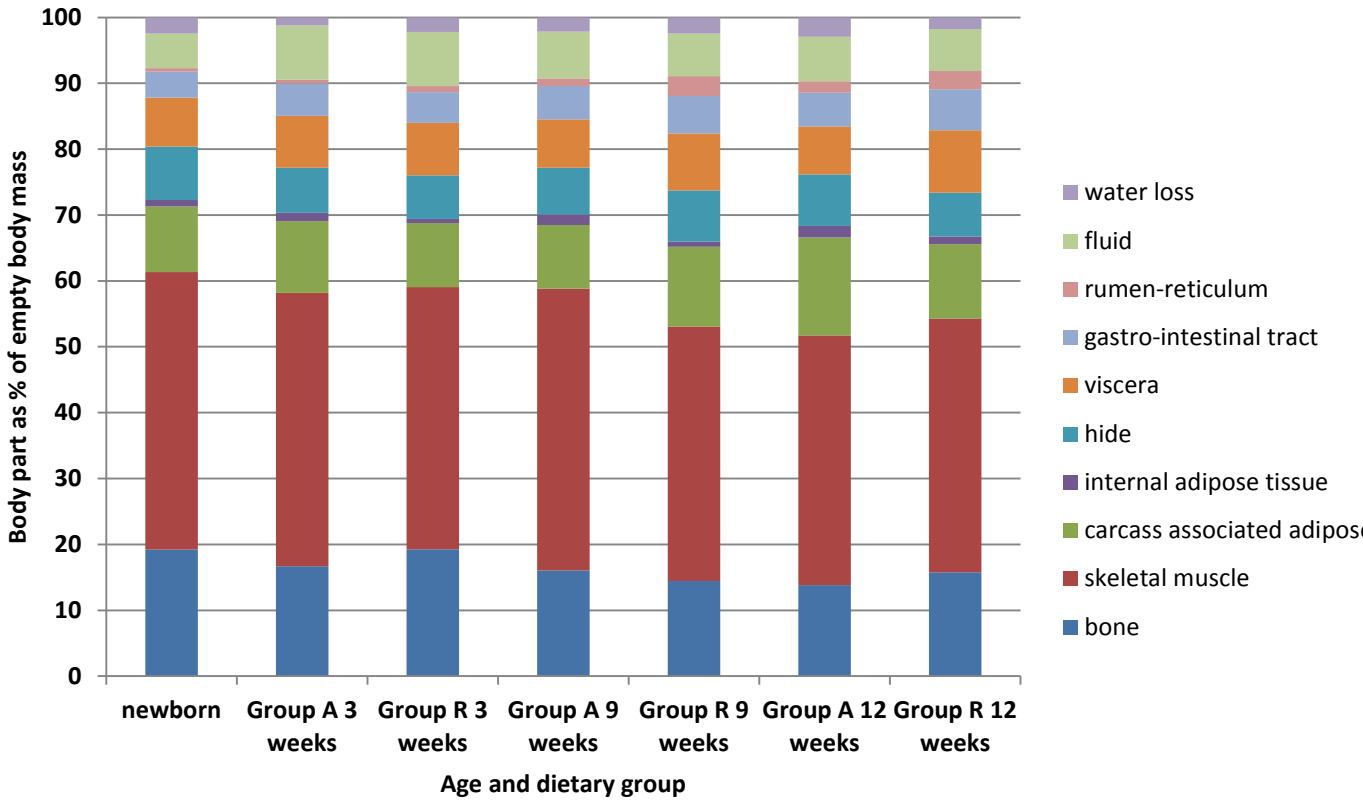

Figure 4.4: Mean carcass composition as percentage of empty body mass of newborn, 3 week, 9 week and 12 week old Holstein bull calves in both Group A and Group R (*n* = 3 per group). Empty body mass = Total body mass less gastro-intestinal tract contents. (Gastro-intestinal tract = total empty GIT less rumen-reticulum, viscera = all organs, urogenital tract, testicles, brain, eyes and spinal cord, fluid = blood, urine and any other collected fluids).

Figure 4.5: Gross visual inspection of the interior surface of the rumen in 9 week old calves from **a)** Group A and **b)** Group R.

4.4 Discussion

Due to the high value of Holstein heifer calves as potential replacement milking animals within the dairy herd, this study assessed the carcass composition of bull calves at various time points throughout the first 12 weeks of life. Conclusions taken from bull calves in this study may be related to those of heifer calves of the same age, as during this pre-pubertal period of life, no differences in carcass composition due to gender are expected.

Analyses of spiral CT images of calf carcasses in this study were a quick and easy method of describing the composition of bull calves. This technique was less time consuming than traditional hand dissection techniques in which an investment of at least 24 working hours would be required for total half carcass dissection. Furthermore, analyses of carcass composition using CT technologies are more reliable and precise than hand dissection (Kongsro et al., 2008).

Specific gravity of colostrum, plasma total protein concentration for calves at 48 hours of age (where applicable) and body weight and morphometric measures for calves prior to euthanasia were comparable to those recorded during a larger heifer calf study conducted under the same husbandry conditions (Table 4.2). However, there were statistical differences between Group A and R in terms of body weight, withers height and BCS in the larger heifer study (Thesis Chapter 3); this was not always the case for the current bull calf study. There were no statistical differences between Group A and Group R bull calves in BCS at all ages, and body weight or withers height at 9 and 12 weeks. The lack of statistical difference in these measures is likely due to the small sample size in the current study.

The provision of restricted milk or MR for dairy calves is commonplace on U.K. dairy farms (Thomas et al., 2001), and has been justified by anecdotal or short term evidence (Anderson, 2011). Arguments against *ad libitum* MR feeding include the perceived risk for increased adipose tissue deposition following provision of increased energy through MR. In the current study, significant differences in internal adipose deposition between Group A and R were determined at all ages. While internal adipose as a percentage of empty body mass increased for Group A calves from birth to 3 weeks, it decreased for Group R animals. Internal adipose tissue continued to increase with age for both dietary groups from 3 weeks of age onwards. Although there were significant dietary group differences in terms of

internal adipose tissue deposition at all ages, this adipose depot only represented approximately 10% of total body adipose tissue in all animals. The majority of total body adipose tissue was carcass associated (largely inter and intra-muscular) and although there was a tendency for this to increase with age, obvious differences were not discernible between dietary groups (Table 4.3, Figures 4.2 and 4.3).

The results gathered from this study indicate that internal adipose tissue is mobilised preferentially over carcass associated adipose tissue during periods of insufficient dietary energy intake during the first 3 weeks of life in calves fed restricted MR. A study conducted by Brown *et al.* (Brown et al., 2005) reported that Holstein heifers calves fed increased protein and energy from 2 to 8 weeks and euthanased at 8 weeks of age did not have significantly different carcass composition to heifers fed a standard energy ration. Brown's study examined only the carcass of heifer calves and not the viscera or associated adipose depots which would complement the findings of the current study.

It is well known that early intake of concentrate feed promotes rumen development (Anderson et al., 1987). Studies have shown that concentrate feed intake is greater in restricted milk fed calves compared to *ad libitum* milk fed calves during the first few weeks of life, although no differences in post-weaning intakes have been reported (Andreia De Paula et al., 2008; Borderas et al., 2009a; Jasper and Weary, 2002). In Chapter 3 of this thesis, concentrate intake was negligible in heifer calves fed restricted MR until approximately 3 - 4 weeks of age. This resulted in minimal growth and loss of BCS compared to their *ad libitum* MR fed counterparts. This would suggest that promotion of rumen growth by restricting milk intakes has an adverse impact on early life growth. Veal calves are often fed *ad libitum* milk replacer, with or without small amounts of concentrate feed, in order to ensure sufficient Average Daily Gain (ADG) in weight prior to finishing. This practice reduces the opportunity for rumen development (Webb et al., 2012). The present study supports previous evidence that rumen development of restricted MR fed calves is greater than that of similar aged *ad libitum* fed calves (Baldwin et al., 2004) due to increased concentrate intake from an earlier age (Quigley et al., 2006), as demonstrated by the significantly greater rumen-reticulum weights in restricted MR fed calves at 3, 9 and 12 weeks. These differences are a reflection of the concentrate intakes observed during a larger intervention study of heifer calves (Chapter 3). Concentrate intakes during the heifer

study were negligible for both dietary groups from birth to 3 weeks but were significantly greater in Group R compared to Group A calves until just prior to weaning.

Although appropriate rumen development is undoubtedly important in ensuring sufficient energy intake and utilisation in the dairy calf (Coverdale et al., 2004), the age at which this must occur by has not been documented. In practical terms, *ad libitum* MR feeding of dairy calves ensures all energy requirements are met through liquid feed. The transition from a liquid based to a solid diet must occur gradually, enabling sufficient consumption of concentrate feed and minimal impact on growth rates.

This study assessed the effect of increased MR feeding on body composition and live growth. However, further work utilising more animals is necessary to assess the full effects. Recent developments in technology can now allow for CT-analysis of live, anaesthetised animals; this would allow a larger number of animals to be studied. It would also allow for the study of heifer calves, in which case the effect of neonatal diet on early mammary development may be studied in live animals.

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Chapter 5

Assessment of glucose metabolism and insulin sensitivity in the Holstein dairy calf

5.1 Introduction

Today's dairy industry is shaped by increasing production costs and environmental pressures and decreasing farm gate milk prices (Guardian, 2015; Hutjens, 2011). In this climate, profitability is most readily achieved using high yielding breeds of cattle. The genetic capacity of the Holstein dairy cow in terms of milk production is huge (up to 32,000 litres in 365 days (Holstein Association USA, 2014)), but full expression of this potential is dependent upon appropriate husbandry and nutrition (Boichard and Brochard, 2012). The mean annual milk yield of UK Holstein cattle has increased from 5398 litres in 1995 to 7327 litres in 2012 (DEFRA, 2014). However, this 36% increase in recorded productivity remains well below the achievable capabilities of the breed, suggesting that current husbandry and nutrition remain limiting factors for milk production in the UK.

In metabolic terms, the Holstein cow works at approximately four-fold maintenance levels during peak lactation. As a consequence of this extreme nutrient partitioning towards milk production, dairy Holsteins spend a considerable proportion of their productive lives in a state of negative energy balance (NEBAL). At peak lactation, it is estimated that metabolic requirements for amino acids, glucose and fatty acids are increased by two, three and five fold over maintenance values (Bell, 1995). The partitioning of nutrients for milk production on this scale increases the risk of metabolic disease and reduced fertility, both of which are important issues relating to poor performance in the modern dairy cow (Lucy, 2001). Common diseases associated with NEBAL in the lactating Holstein include ketosis, fatty liver disease, hypocalcaemia and displaced abomasum (Goff and Horst, 1997). The pathogenesis of these conditions has been associated with markedly increased rates of lipid mobilisation from adipose tissues as a metabolic response to reduced appetite and energy intake during a crucial period of high glucose demand (Allen and Piantoni, 2013).

Insulin resistance is defined as a state in which an increased quantity of insulin is required to elicit a normal response to promote glucose homeostasis (Lebovitz, 2001). Dairy cows generally demonstrate a degree of insulin resistance as a physiological consequence of energy partitioning during late gestation and early lactation in order to support foetal growth and mammary function (Hayirli, 2006; Holtenius and Holtenius, 2007). Insulin resistance in adipose tissue is associated with increased lipolysis and decreased lipogenesis,

thus mobilisation of fat reserves occur, increasing circulating NEFA concentrations. This phenomenon is clearly documented not only in dairy cattle but also in other species (Faulkner and Pollock, 1990; Johnson, 2008). The magnitude of insulin resistance in dairy cattle has been associated with dry matter intake during the transition period (Pryce et al., 2004). To minimise the potential negative effects of insulin dysregulation, nutritional and management strategies should be optimised to limit both increases in body condition score (BCS) and decreases in feed intake to support metabolic changes that may occur during this time (Ingvartsen, 2006).

While nutrition, particularly during the 'dry period' is important in the prevention of energy imbalance, it is increasingly recognised that pre-natal and neonatal nutrition may impact on adult metabolism (Van Amburgh et al., 2011). Some of the phenotypic variation (e.g. body weight and height, milk yield etc.) between individual animals has been attributed to early life, epigenetic factors (Funston and Summers, 2013; Heijmans et al., 2008; Singh et al., 2010). Similarly, there is evidence that nutrient availability during early life is associated with future health and performance of dairy calves. Many studies have reported increased growth rates (e.g. 0.8 kg *vs*. 0.2 kg/day) (Appleby et al., 2001; Jasper and Weary, 2002) for calves fed at higher planes of nutrition with subsequent benefits to future health and performance (Anderson, 2008; Blome et al., 2003; Drackley; Jasper and Weary, 2002; Morrison et al., 2012; Soberon et al., 2012; Van Amburgh).

Under natural conditions calves could be expected to suck their dams approximately 8 to 10 times per day, consuming small volumes (approximately 1-2 litres) of milk at each meal (Borderas et al., 2009). By contrast, most modern dairy calves are limit fed and receive milk or milk replacer in discrete meals of between 1.5 and 3.0 litres which are generally provided twice daily (Appleby et al., 2001; Drackley, 2008; Jasper and Weary, 2002). This twice daily feeding system has recently been expanded to include increased volumes of milk (up to 4 litres) per meal to promote early growth (Bach et al., 2013). However, evidence suggests that this relatively increased feeding regimen impacts negatively on glucose/insulin dynamics (Bach et al., 2013; Hostettler-Allen et al., 1994). The impact of insulin dysregulation in youngstock on future metabolic health is largely unknown but it may be important.

Glucose/insulin interactions have been evaluated using a variety of dynamic tests: by assessing the insulin response to glucose (Glucose Tolerance Test, GTT) or tissue responsiveness to insulin (Insulin Sensitivity Test, IST). These simple and practical tests have been used widely in both human and veterinary medicine (Eiler et al., 2005; Holtenius and Holtenius, 2007; Pildes et al., 1969) and are dependent on the evaluation of standard variables (basal and peak glucose concentrations, glucose clearance rates, time to nadir and area under the glucose curve) following the administration of calibrated doses of exogenous glucose and/or insulin. More recently a Combined Glucose Insulin Tolerance (CGIT) test has been developed to facilitate the concurrent evaluation of both insulin responsiveness and sensitivity. This method has been validated for use in the horse for which the resulting data have greatly improved both the understanding and practical management of insulin dysregulation (Eiler et al., 2005). To date the CGIT has not been applied to evaluate glucose-insulin dynamics in the dairy calf. It is possible that an understanding of early life carbohydrate handling capabilities may allow prediction of future metabolic health in Holstein dairy calves.

This study was split into two smaller, linked studies. The objective of Study 1 was to evaluate the use of a CGIT test in 2 week old pre-ruminant Holstein dairy calves. The hypothesis, that *ad libitum* MR fed calves would be relatively more insulin resistant than those calves fed restricted MR quantities was tested. The objective of Study 2 was to identify differences in the insulin sensitivity and glucose homeostasis response via the use of the combined test in the same calves tested at 2, 12 and 39 weeks of age.

5.2 Materials and methods

Animals and study design

Holstein dairy heifer calves born between January and April 2012 were recruited onto the study. The calves were produced by primiparous (*n* = 4) or multiparous (*n* = 8) dams, within a year-round calving dairy herd (University of Liverpool's Wood Park Dairy Farm, Neston, Wirral, U.K. 53^{o}N). Only single born calves with no evident health complications were accepted. For Study 1, animals were tested at 2 weeks of age; for Study 2, the same animals were re-tested at 12 and 39 weeks of age.

The study compared calves which had been assigned prior to birth to one of two milk replacer (MR) feeding strategies: Group A; *ad libitum* MR access (*n* = 6) or Group R; restricted MR access (*n* = 6), Table 5.1.

Table 5.1: Nutritional and husbandry protocols used for calves in the *ad libitum* milk replacer (MR) access and restricted MR access groups from birth until 12 weeks of age.

Group	Milk replacer allowance	Milk replacer feeding method	Weaning protocol	Housing method	Concentrate feeds and forage
A (*ad libitum* MR)	*Ad libitum* access until day 63	Automatic teat feeder	Stepwise restriction of daily MR allowance over 21 days	Group housed from birth (n ≤ 6)	*Ad libitum* access to grass hay and up to 2.5 kg concentrate feed (coarse mix) daily
R (restricted MR)	5L daily until day 21, then 6L daily until day 56 (provided as 2 equal meals, (09:00 & 17:00hrs)	Individual bucket to day 21, thereafter group trough fed	50% reduction of MR allowance over 7 days.	Individually housed until 21 days then group housed (n ≤ 6)	*Ad libitum* access to grass hay and up to 2.5 kg concentrate feed (coarse mix) daily

Animal Husbandry

Birth to 12 weeks

Calves were born into group accommodation with between 5 and 15 cows present. For daytime calvings (08:00 and 18:00 hours), calves were removed from their dams within 4 hours *post-partum*. Calves born between 18:00 and 08:00 hours remained with their dam for up to 12 hours.

Sequentially born calves were assigned to rearing-groups (n ≤ 6), such that the ages of individual animals in each group ranged by no more than 14 days. Alternate rearing-groups were pre-designated to receive one of two milk replacer (MR) feeding strategies until targets were met: Group A; *ad libitum* MR access (*n* = 6) or Group R; restricted MR access (*n* = 6, Table 5.1).

Between 3 and 4 litres of colostrum from the calves own dam (collected as soon as possible after birth) were administered to each calf by oesophageal feeders. Further freshly-collected, dam-specific transitional colostrum meals were individually bucket fed to all calves twice daily (2 - 2.5 litres per meal) for 4 days.

All calves were offered a proprietary MR from the same production batch throughout the study (96.97% DM, 22.17% crude protein, 19.76% oil, 7.02% ash, ME 21.57 MJ/kgDM, pH 5.96, Blossom Easy Mix, Volac, Hertfordshire, U.K.). Powdered MR was thoroughly mixed with water (125g MR/litre, 37^{o}C) immediately prior to feeding. Group A calves were trained to the computerised teat feeder (Vario feeder, Forster Technik, Germany) from which *ad libitum MR* was dispensed from birth. Milk replacer was therefore available to Group A calves throughout the initial 4 day colostrum feeding period. Daily MR intakes for individual Group A calves were automatically recorded (± 0.1 L). In accordance with routine farm management, Group R calves were offered MR from day 4, predefined as 5 litres daily until 3 weeks of age, then 6 litres daily thereafter until weaning onset at 8 weeks. Calves were offered MR twice daily split into two equal meals and fed at 09:00 and 17:00 hrs (Table 5.1). The age and timing of weaning from MR differed between the 2 dietary groups. Group R calves began weaning at 8 weeks of age by reduction of MR provision to 3 litres once daily, fed at 09.00 hrs for 1 week until complete cessation of MR feeding at 9 weeks. Calves in Group A began weaning at 9 weeks with restriction to 16 litres MR daily, progressive further

restriction followed by mean reduction by 0.75 litres daily until completion of weaning at 84 days of age was carried out (Figure 5.1).

Concentrate feedstuffs (Primestart coarse mix, 86.2% DM, 18% crude protein, 8% crude fibre, 9.5% ash, 3.5% oil, ME 14.46MJ/Kg, BOCM Pauls Ltd U.K.) were available to all calves (to a maximum intake of 2.5 kg per head) throughout the first 12 weeks. All calves had *ad libitum* access to forage (grass hay and wheat straw bedding) and fresh water but intake rates were not recorded for these.

From birth to 21 days of age, calves in Group R were individually housed in metal gated pens (1m x 2m), over slatted flooring and bedded with wheat straw. At 21 days of age, Group R calves were moved to deep, wheat straw-bedded, group pens (5m x 6m; $n \leq 6$, age range ≤ 14 days). Group A calves were grouped by age ($n \leq 6$, range ≤14 days) but were directly introduced to group pens (as above) from birth.

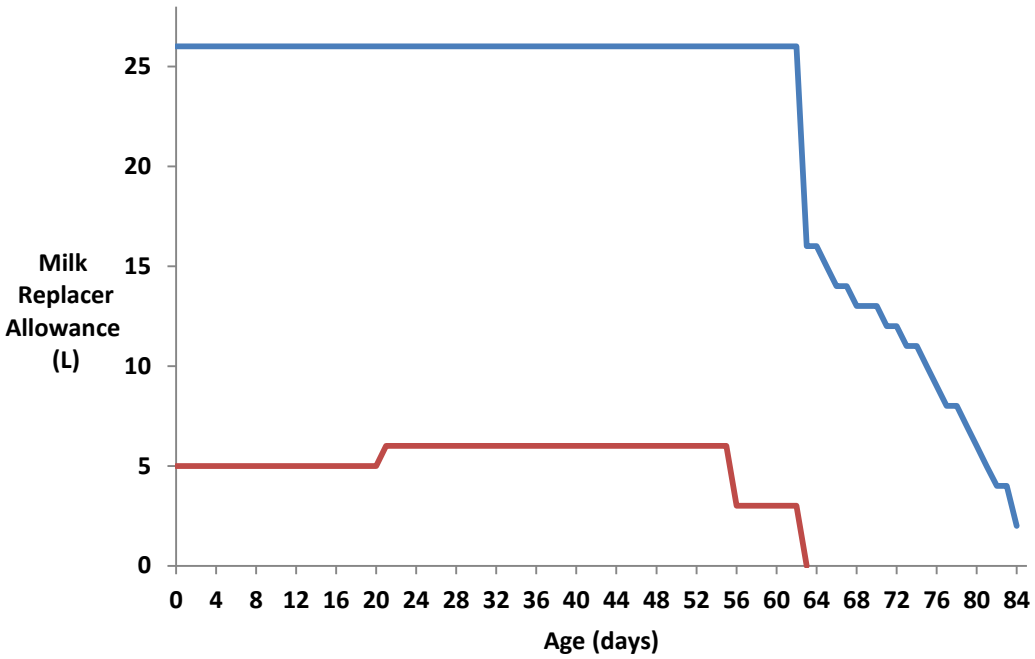

Figure 5.1: Daily milk replacer (MR) allowance (litres/day) for calves in Group R (red line, *n* =6) and Group A (blue line, *n* =6) from birth to weaning. Group R began weaning at 56 days by reduction of MR from 6 litres to 3 litres for 7 days prior to cessation of MR feeding. Group A began weaning at 63 days by a gradual 'step-down' beginning at 16 litres.

From 12 weeks of age

From 12 weeks of age onwards, calves in both dietary groups were subject to common nutritional and husbandry protocols. Irrespective of dietary group, at 16 weeks of age all calves were transferred to follow on accommodation (indoor straw yards 18m x 6m, approximately *n*=12 per group). From 12 to 20 weeks of age, the diet consisted of a maximum of 2.5kg of concentrate feed per head (86.20% DM, 18.00% crude protein, 4.00% oil, 9.50% ash, 12.50% crude fibre , ME 14.55 MJ/Kg Super Rearer 18 nuts, BOCM Pauls, U.K.) and *ad libitum* grass hay, with fresh water available at all times.

In accordance with standard farm practice, nutrition from 20 weeks onwards was highly variable consisting largely of refusals from the total mixed rations fed to the lactating, far off dry cows and transition dry cows. However, depending on the amount of refusals each day, additional maize silage or grass silage was added to the diet.

Test Procedure

Study 1, Comparative evaluation of the CGIT, GTT and IST in two week old Holstein heifer calves with respect to pre-weaning nutrition: Evaluation of individual animal responses to each of the 3 dynamic tests (GTT, IST, CGIT) when each calf had reached 2 weeks (+/- 2 days) of age. Test orders were randomly ascribed for each calf, and two clear days were allowed between successive tests.

Study 2, Age-related changes in glucose-insulin dynamics: Evaluation of the responses to the CGIT at 2, 12 and 39 weeks of age.

Studies 1 and 2: On the day prior to the onset of the first test, an indwelling jugular vein catheter (Vygon, U.K.), was placed into the left side jugular vein of each calf under local anaesthetic and aseptic conditions. To maintain patency, catheters were flushed daily with heparinised saline (200IU/ml heparin, 0.9% NaCl solution). Animals were fasted for 12 hours prior to the onset of each test. For both studies, all tests began between 08:30 and 09:00hrs. The body weight (± 0.5 Kg) of each calf was recorded 30 minutes prior to each test

onset in order to calculate doses of glucose and/or insulin for infusion. Blood samples (5ml) were collected into lithium heparin tubes (BD Vacutainer, Becton Dickinson and Co., U.S.A.) at times -10 (T_{-10}), -5 (T_{-5}) and 0 (T_0) minutes prior to infusion of glucose (GTT, Study 1), insulin (IST, Study 1) or both glucose and insulin (CGIT, Studies 1 and 2) to allow the determination of mean fasted plasma glucose (mmol/L),NEFA (mmol/L) and insulin (µg/L) concentrations. Following rapid intravenous administration of the glucose (GTT, 150mg/kg, 40% glucose, Dales Pharmaceuticals, U.K.), insulin (IST, 0.05 U/kg insulin, Humulin® R, U.S.A.), or combined challenge (CGIT, 150 mg/kg, 40% glucose, 0.05 U/kg insulin) (Table 5.2). Catheters were immediately flushed with 20ml of sterile saline and further blood samples were collected at 1 (T_1), 5 (T_5), 10 (T_{10}), 15 (T_{15}), 25 (T_{25}), 35 (T_{35}), 45 (T_{45}), 60 (T_{60}), 75 (T_{75}), 90 (T_{90}), 105 (T_{105}), 120 (T_{120}), 135 (T_{135}), and 150 (T_{150}) minutes for all tests. Blood samples were immediately placed on ice prior to centrifugation (2000g for 15 minutes at 4°C). Plasma was harvested and immediately aliquoted into 2ml microtubes (Axygen Scientific, California) and stored at -20°C pending analysis.

Table 5.2: Steps involved and doses required for the 3 tests carried out after fasting blood samples (-10, -5 and 0 mins) were taken, each step follows on immediately from the previous, and the 1 minute sample is taken after the final step outlined in the table.

Test	**Step 1**	**Step 2**	**Step 3**
CGIT	Glucose 150mg/kg	Insulin 0.05 U/kg	20ml sterile saline
GTT	Glucose 150mg/kg	20ml sterile saline	
IST	Insulin 0.05 U/kg	20ml sterile saline	

Plasma glucose concentrations for all samples were determined in duplicate using the hexokinase method (KoneLab 30i; Thermo Fisher Scientific, Finland; limit of detection, 0.1mmol/L: range, 0.3 to 40.0 mmol/L; inter and intra-assay coefficients, 4.2 and 1.0%). Plasma NEFA concentrations using the colormetric method for all time points (T_0 - T_{150}) (Randox Biochemistry Analyzer; NEFA Assay; inter and intra-assay coefficients, 2.3 and 6.0%) and plasma insulin concentrations atT_0, T_{45} and T_{75} were also determined (Mercodia Bovine Insulin ELISA, Kit number: 10-1201-01, Mercodia Inc., Sweden; inter and intra-assay coefficients 5.3 and 6.7%, limit of detection 1 mU/L).

Statistical analyses

All analyses were carried out using STATA 13 (StataCorp LP, USA). Data were tested for normality using the Shapiro-Wilk W test. Mean baseline concentrations for glucose, NEFA and insulin, peak and minimum glucose and NEFA concentration and time to return to baseline glucose concentrations were calculated where appropriate and simple univariable analyses, initially using students t tests were carried out to investigate any differences associated with dietary group for each test at each age.

Area under the curve was calculated for glucose (AUC_g) for the 3 tests during Study 1, and for glucose, NEFA (AUC_n) and insulin (AUC_i) during Study 2 using the trapezoidal method. AUC was compared between dietary groups and test ages using student's t tests.

Since measurements were clustered within calves, random effects linear regression models with calf identity as a random effect were employed for glucose, NEFA or insulin concentrations (outcome variables). Explanatory variables offered to the models were: time during the test, with an interaction with dietary group.

5.3 Results

All animals remained healthy throughout each test and no overt signs of hypoglycaemia were noted at any time. Data were analysed with respect to plasma glucose concentrations throughout the 3 tests for Study 1, and plasma glucose, NEFA and insulin concentrations throughout tests in Study 2.

Study 1: Comparative evaluation of the CGIT, GTT and IST in two week old Holstein heifer calves with respect to pre-weaning nutrition.

At the outset of the first test, the mean age of calves (*n* = 12) was 12.4 days (range 9 - 17 days). There were no dietary group differences in body weight (mean: 43.4 kg, 95% CI 41.9 - 45.0, P = 0.423).

Glucose concentrations: The mean fasting baseline plasma glucose concentrations (pre-infusion) were similar between dietary groups and for each of the 3 tests (mean: 5.54 mmol/L, 95% CI 5.30 - 5.79, P > 0.05, Table 5.3).

Glucose infusion resulted in the rapid development of a hyperglycaemic surge for both the GTT and CGIT tests with maximal plasma glucose concentrations recorded in samples collected 1 minute post infusion (mean: 9.40 mmol/L, 95% CI 9.07 - 9.74). Plasma glucose concentrations subsequently decreased and had returned to baseline by 52.2 minutes (95% CI 35.2 - 69.1) for the GTT and 19.8 minutes (95% CI 14.1 - 25.6) for the CGIT test.

In contrast to the GTT, changes in plasma glucose concentrations following the dual insulin and glucose infusion of the CGIT test were bi-phasic. After returning to baseline concentration at 19.8 minutes, plasma glucose concentrations continued to decrease and attained a nadir at 2.60 mmol/L (95% CI 1.94 - 3.25) 41.7 minutes (95% CI 36.2 - 47.1) post-infusion for Group A and 65.0 minutes (95% CI 41.3 - 88.7) post-infusion for Group R animals (P = 0.020). Plasma glucose concentrations subsequently increased progressively for all calves post-nadir, but only 3 out of the 12 calves (all Group A) had returned to baseline plasma glucose concentrations by the end of the test at 150 minutes post-infusion.

During the IST test, infusion of insulin was associated with the immediate onset of a progressive decrease in plasma glucose concentration. A nadir was reached at a mean value of 1.87 mmol/L (95% CI 1.46 - 2.28) 35 minutes (95% CI 31.2 - 38.8) after insulin infusion for all calves. Glucose concentrations increased from 35 minutes to the termination of sampling (150 minutes post-infusion). However, only 3 animals had returned to pre-test, baseline values for plasma glucose concentration by the final sampling point, the mean plasma glucose concentration at 150 minutes post-infusion was 5.18 mmol/L (95% CI 4.73 - 5.62).

For all 3 tests, the mean AUC_g was similar for each dietary group (Table 5.4).

The explanatory variable in the final multivariable plasma glucose concentration model for the GTT, IST and CGIT tests was time during the test with an interaction with dietary group (full model output, Appendix D, Table D.1- D.3). Predicted marginal mean glucose concentrations derived from the regression models are presented for all 3 tests (Figure 5.2).

Table 5.3: Mean (95% CI) plasma glucose concentrations throughout the GTT, IST and CGIT tests for calves in Group A (*n* = 6) and Group R (*n* = 6) at 2 weeks of age.

Time (mins)	Mean plasma glucose concentration (mmol/L, 95% CI)								
	GTT			IST			CGIT		
	Group A	Group R	P value	Group A	Group R	P value	Group A	Group R	P value
Baseline	5.62 (4.81 - 6.29)	5.48 (5.15 - 5.85)	0.439	5.71 (4.66 - 6.65)	5.36 (5.00 - 5.72)	0.345	5.88 (4.91 - 6.84)	5.34 (4.11 - 6.58)	0.201
1	9.48 (8.21 - 10.76)	9.63 (9.17 - 10.08)	0.397	5.64 (4.67 - 6.63)	5.40 (4.95 - 5.85)	0.273	9.58 (8.80 - 10.36)	8.93 (8.33 - 9.53)	0.060
5	8.50 (7.60 - 9.40)	8.59 (8.46 - 8.72)	0.400	5.15 (3.94 - 6.36)	4.50 (3.78 - 5.22)	0.125	8.41 (7.72 - 9.09)	7.60 (7.02 - 8.18)	0.022
10	8.00 (7.15 - 8.85)	7.88 (7.52 - 8.23)	0.367	4.25 (3.06 - 5.42)	3.87 (3.27 - 4.40)	0.230	6.67 (4.73 - 8.60)	6.44 (5.74 - 7.15)	0.333
15	7.49 (6.55 - 8.44)	7.33 (6.50 - 8.15)	0.370	3.48 (2.42 - 4.53)	3.25(2.61 - 3.86)	0.320	5.78 (4.92 - 6.63)	5.33 (4.51 - 6.15)	0.180
25	6.48 (5.48 - 7.50)	6.27 (5.35 - 7.19)	0.340	2.40 (1.41 - 3.39)	2.15 (1.49 - 2.78)	0.300	4.03 (2.96 - 5.11)	3.82 (2.76 - 4.87)	0.360
35	5.82 (4.73 - 6.90)	5.58 (4.54 - 6.61)	0.344	2.20 (1.37 - 2.98)	1.87 (1.22 - 2.49)	0.211	3.15 (2.04 - 4.26)	2.83 (1.66 - 4.00)	0.313
45	5.60 (4.53 - 6.69)	5.16 (4.27 - 6.05)	0.213	2.65 (1.81 - 3.49)	2.25 (1.81 - 2.64)	0.150	2.93 (1.64 - 4.23)	2.48 (1.37 - 3.60)	0.257
60	5.47 (4.79 - 6.15)	4.75 (4.25 - 5.25)	0.027	3.15 (2.21 - 4.06)	2.62 (2.07 - 3.17)	0.117	3.93 (2.51 - 5.34)	2.73 (1.80 - 3.67)	0.050
75	5.49 (4.90 - 6.08)	4.44 (3.99 - 4.90)	0.002	3.70 (2.52 - 4.84)	3.28 (2.57 - 3.91)	0.222	4.46 (2.70 - 6.21)	2.98 (2.08 - 3.87)	0.041
90	5.34 (4.64 - 6.06)	4.43 (3.98 - 4.87)	0.009	4.23 (3.04 - 5.39)	3.58 (2.83 - 4.32)	0.131	4.74 (3.08 - 6.40)	3.20 (2.40 - 4.00)	0.028
105	5.36 (4.60 - 6.12)	4.61 (4.23 - 4.99)	0.023	4.56 (3.44 - 5.68)	4.18 (3.20 - 5.12)	0.261	5.18 (3.49 - 6.87)	3.48 (2.60 - 4.36)	0.022
120	5.41 (4.61 - 6.22)	4.82 (4.31 - 5.32)	0.068	4.98 (3.81 - 6.09)	4.43 (3.65 - 5.19)	0.167	5.41 (3.75 - 7.07)	3.71 (2.81 - 4.60)	0.022
135	5.50 (4.64 - 6.36)	4.82 (4.50 - 5.14)	0.043	5.20 (4.19 - 6.17)	4.90 (4.10 - 5.65)	0.275	5.63 (3.99 - 7.27)	4.17 (3.42 - 4.91)	0.032
150	5.39 (4.58 - 6.22)	4.79 (4.42 - 5.17)	0.056	5.28 (4.33 - 6.18)	5.12 (4.55 - 5.63)	0.351	5.72 (4.14 - 7.29)	4.48 (3.59 - 5.38)	0.055

Table 5.4: Area under the glucose curve (AUC_g) for both Group A and Group R animals during the GTT, IST and CGIT tests at 2 weeks of age.

Test	Area under the glucose curve (AUC_g, mmol/L/min) (95% CI)		
	Group A	**Group R**	**P value**
GTT	939.6 (823.6 - 1055.6)	851.1 (798.5 - 903.7)	0.052
IST	648.6 (493.8 - 803.5)	582.6 (505.0 - 660.1)	0.175
CGIT	338.4 (245.2 - 431.7)	284.6 (226.5 - 342.8)	0.119

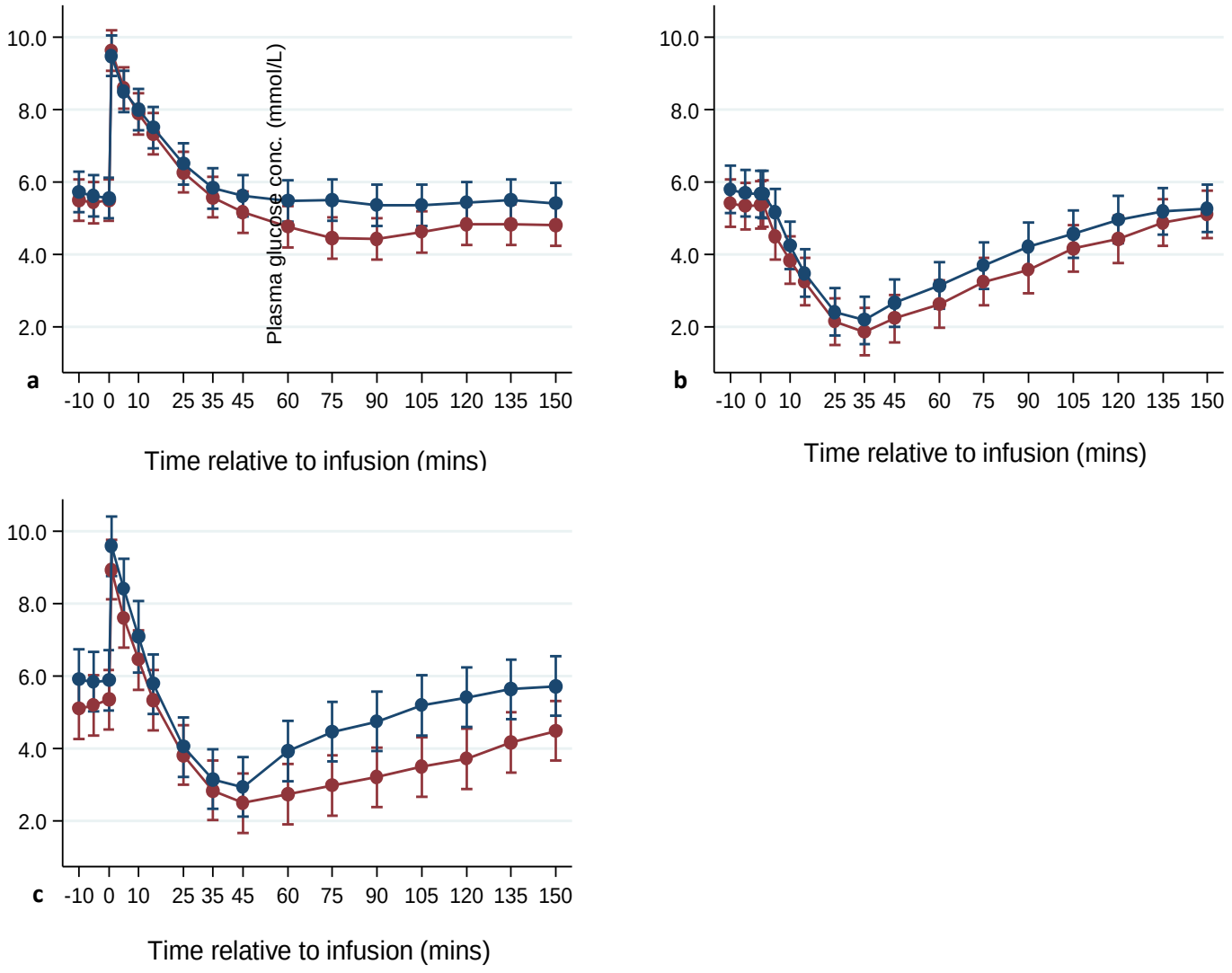

Figure 5.2: Marginal means (95% CI) of predicted plasma glucose concentration (mmol/L) for calves in Group A (*n* = 6, blue line) and Group R (*n* = 6, red line) throughout a) the GTT, b) the IST and c) the CGIT test at 2 weeks of age.

Study 2: Age-related changes in glucose-insulin dynamics.

The mean age of animals was 12.1 weeks (95% CI 11.8 - 12.4) at the 12 week CGIT test and 39.3 weeks (95% CI 37.3 - 39.2) at the 39 week test. The body weight of animals differed between dietary groups at the 12 week test (Group R: 96.3 kg, 95% CI 85.8 - 106.7; Group A: 115.2 kg, 95% CI 99.1 - 131.3, P = 0.015), but did not differ at the 39 week test (mean 237.9 kg, 95% CI 203.9 - 271.9).

Glucose concentrations: Data pertaining to plasma glucose concentrations during the 2 week CGIT test are presented in Study 1.

During the 12 week CGIT test, the mean baseline plasma glucose concentration prior to glucose and insulin infusion was 5.11mmol/L (95% CI 4.83 - 5.38) and was not significantly different to baseline values at 2 weeks of age (P = 0.160, Table 5.5). However, by 39 weeks, the mean baseline plasma glucose concentration was 4.41 mmol/L (95% CI 4.06 - 4.75), significantly lower than at 2 and 12 weeks (P < 0.001).

Glucose (and insulin) infusion resulted in the rapid development of a hyperglycaemic surge for CGIT tests at all 3 ages. Maximal plasma glucose concentrations were recorded in samples collected 1 minute post infusion. During the 12 week test, the maximal plasma glucose concentration was 10.66 mmol/L (95% CI 9.97 - 11.35), which was significantly greater than that recorded at 2 weeks of age (9.26 mmol/L, 95% CI 8.80 - 9.71, P < 0.001). Furthermore, the maximal plasma glucose concentration measured 1 minute post infusion during the 39 week test was 12.14 mmol/L (95% CI 11.05 - 13.23), significantly greater than at 12 weeks (P = 0.009, Figure 5.3).

For CGIT tests at all 3 ages, plasma glucose concentration decreased after the 1 minute sample and had returned to baseline at 37.1 minutes (95% CI 27.4 - 46.8) at 12 weeks and 36.3 minutes (95% CI 24.4 -48.1) at 39 weeks. Time to return to baseline glucose concentration did not differ between the 12 and 39 week tests (P = 0.453), but both were significantly later to return to baseline plasma glucose concentration than at 2 weeks of age (19.8 minutes, 95% CI 14.1 - 25.6, P < 0.02, Figure 5.3).

After returning to baseline, plasma glucose concentrations continued to decrease for the CGIT test at all 3 ages. Differences were highlighted when comparing time to nadir and plasma glucose concentration at nadir between the CGIT tests at the 3 ages.

During the 2 week test, plasma glucose concentration reached a nadir at 2.60 mmol/L (95% CI 1.94 - 3.25) 53.3 minutes (95% CI 40.7 - 65.9) post-infusion. During the 12 week test, a nadir was attained at 3.93 mmol/L (95% CI 3.67 - 4.18), 72.5 minutes (95% CI 60.4 - 84.6) post-infusion. At 39 weeks, a nadir was attained at 2.78 mmol/L (95% CI 2.28 - 3.27), 63.8 minutes (95% CI 45.5 - 82.0) post-infusion.

At 12 weeks, both the plasma glucose concentration and the time at nadir were significantly different to values recorded during the 2 week test ($P < 0.001$ and $P = 0.012$ respectively). However, there were no statistically significant differences between plasma glucose concentration ($P = 0.318$) and time at nadir ($P = 0.156$) between tests carried out at 2 and 39 weeks. Comparison of plasma glucose concentration and time at nadir during the 12 and 39 week tests revealed differences in minimum plasma glucose concentration ($P < 0.001$) but not time to nadir ($P = 0.194$).

Plasma glucose concentrations subsequently increased progressively for all calves during all 3 CGIT tests post-nadir, but only 2 out of the 12 (all Group A) animals at 12 weeks and 3 out of the 12 (all Group A) animals at 39 weeks had returned to baseline plasma glucose concentration by the end of the test at 150 minutes post-infusion (Figure 5.3).

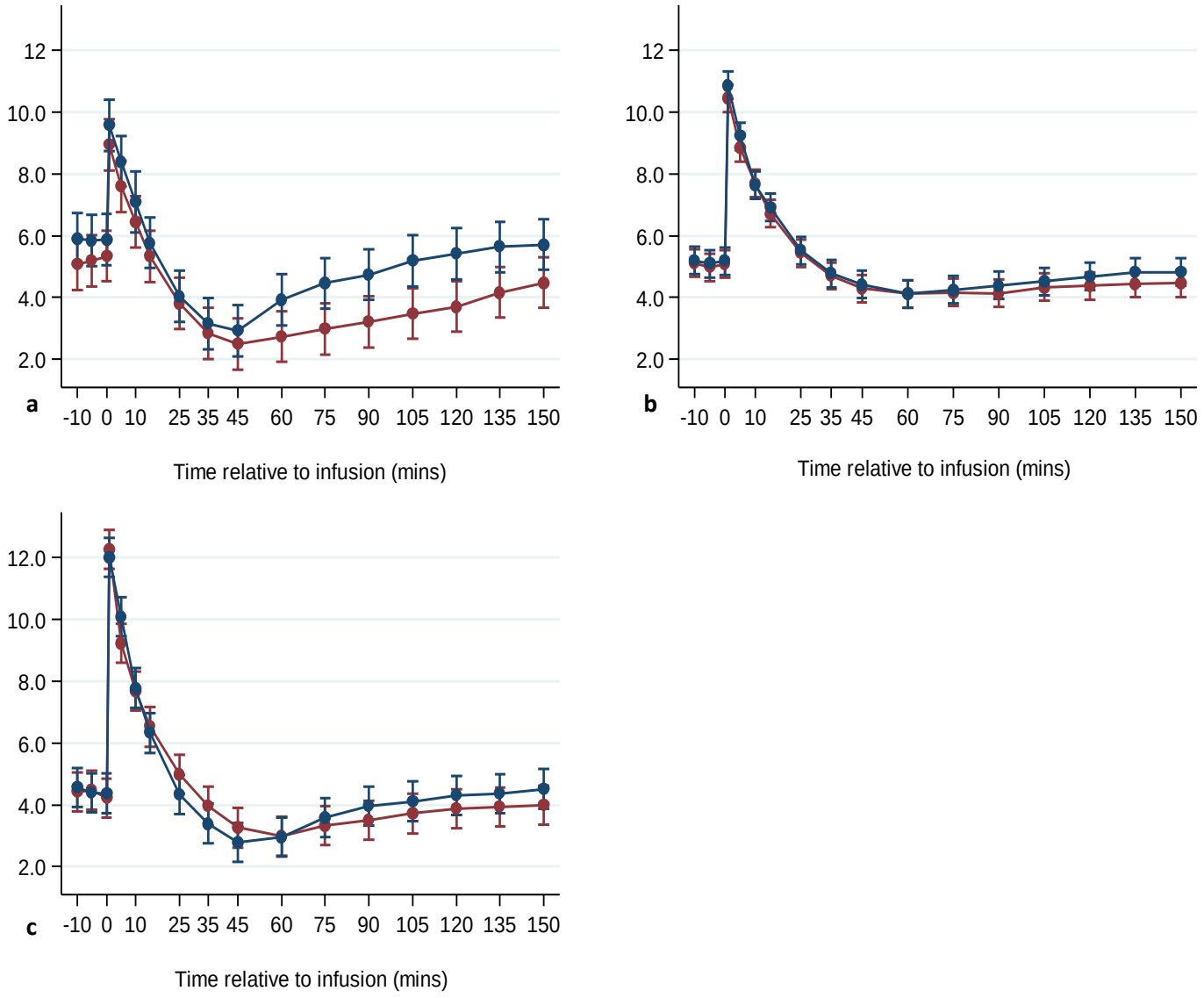

Figure 5.3: Marginal means (95% CI) of predicted plasma glucose concentration (mmol/L) for calves in Group A (*n* = 6, blue line) and Group R (*n* = 6, red line) throughout CGIT tests at a) 2 weeks, b) 12 weeks and c) 39 weeks.

NEFA concentrations: There were no pre-weaning dietary group differences in plasma NEFA concentration throughout any of the CGIT tests.

Mean baseline plasma NEFA concentration was 0.61 mmol/L (95% CI 0.40 - 0.83) at 2 weeks, 1.09 mmol/L (95% CI 0.82 - 1.36) at 12 weeks and 0.70 mmol/L (95% CI 0.37 - 1.02) at 39 weeks (Table 5.5). Values differed significantly between 2 and 12 weeks (P = 0.003) and between 12 and 39 weeks (P = 0.025) but not between 2 and 39 weeks (P = 0.318, Figure 5.4).

Changes in plasma NEFA concentrations in response to infusion of glucose and insulin were biphasic, and were inversely associated with changes in plasma glucose concentrations. This 2 phased response was most clear during the 2 week test.

Plasma NEFA concentrations decreased following glucose and insulin infusion for tests at all 3 ages. During the 2 week test, minimal plasma NEFA concentrations were recorded at 0.16 mmol/L (95% CI 0.03 - 0.30), 21.3 minutes (95% CI 9.51 - 33.0) post-infusion. These values were not significantly different during the 12 week test (0.27 mmol/L, 95% CI 0.17 - 0.36, P = 0.084; 27.7 minutes, 95% CI 14.6 - 40.8, P = 0.215), or the 39 week test (0.15 mmol/L, 95% CI 0.10 - 0.20, P = 0.403; 29.7 minutes, 95% CI 22.2 - 37.1, P = 0.098, Figure 5.4).

During the 2 week test, plasma NEFA concentrations subsequently increased to a maximum of 1.23 mmol/L (95% CI 0.91 - 1.56) at 72.7 minutes (95% CI 45.3 - 100.0).

At 12 weeks, the maximum plasma NEFA concentration was not different to that at 2 weeks (1.40 mmol/L, 95% CI 1.14 - 1.66, P = 0.192), but the time to reach maximal NEFA concentration was significantly greater (108.0 minutes, 95% CI 75.2 - 140.8, P = 0.041). In contrast, at 39 weeks the maximum plasma NEFA concentration was significantly lower than at 2 weeks of age (0.90 mmol/L, 95% CI 0.69 - 1.10, P = 0.032), however time to reach maximum plasma NEFA concentration was not significantly different (75.3 minutes, 95% CI 37.1 - 113.6, P = 0.451). Comparison of maximal plasma NEFA values during the 12 and 36 week tests revealed significantly greater maximal values at 12 weeks than 39 weeks (P = 0.002), but no difference in time to reach maximum values.

During the 2 week test, plasma NEFA concentrations had decreased to 0.77 mmol/L (95% CI 0.60 - 0.95) by the end of the test at 150 minutes. This was not significantly different to the

final plasma NEFA value recorded at 12 weeks (1.00 mmol/L, 95% CI 0.70 - 1.29, P = 0.082), or 39 weeks (0.67 mmol/L, 95% CI 0.52 - 0.83, P = 0.180). However, there were significant differences between final plasma NEFA concentrations at 12 and 39 weeks (P = 0.022, Figure 5.5).

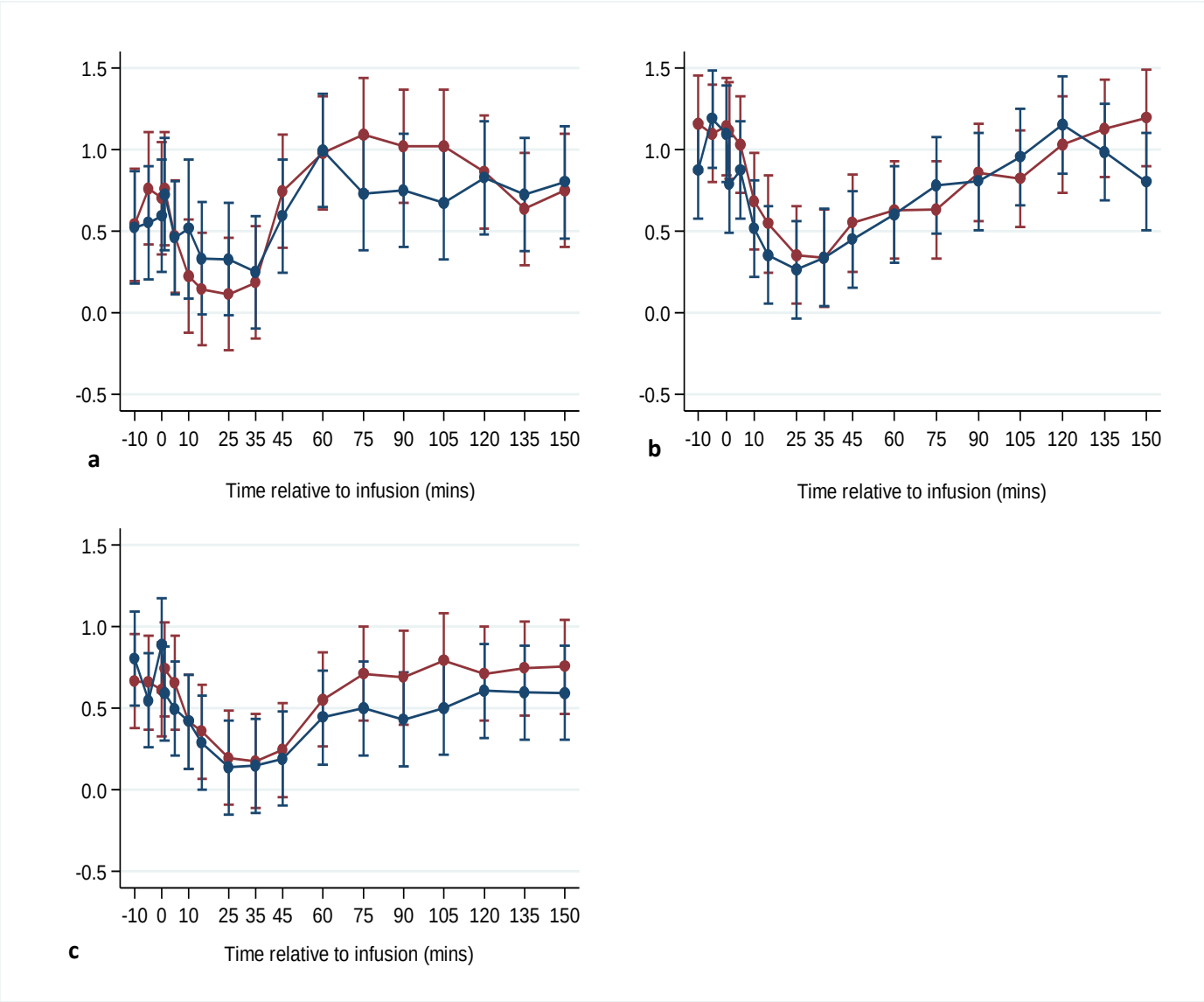

Figure 5.4: Marginal means (95% CI) of predicted plasma NEFA concentration (mmol/L) for calves in Group A (*n* = 6, blue line) and Group R (*n* = 6, red line) throughout CGIT tests at a) 2 weeks, b) 12 weeks and c) 39 weeks.

Insulin concentrations: Plasma insulin concentrations were recorded at baseline, 45 and 75 minutes only.

At the 2 week test, the mean baseline plasma insulin concentration was 0.30 µg/L (95% CI 0.13 - 0.47), this was not significantly different to the baseline concentration at 12 weeks (0.27 µg/L, 95% CI 0.17 - 0.3, P = 0.383), but was significantly different to the baseline concentration at 36 weeks (0.54 µg/L, 95% CI 0.30 - 0.77, P = 0.042). Baseline plasma insulin concentrations were also significantly different between the 12 and 39 week CGIT test (P = 0.016, Figure 5.5).

At 45 minutes post glucose and insulin infusion, plasma insulin concentrations had increased to 1.00 µg/L (95% CI 0.15 - 1.86) during the 2 week test. This was not significantly different to 45 minute plasma insulin concentrations at 12 weeks (0.50 µg/L, 95% CI 0.42 - 0.57, P = 0.103) or at 39 weeks (1.12 µg/L, 95% CI 0.84 - 1.39, P = 0.391). Plasma insulin concentrations 45 minutes post infusion were significantly different between tests conducted at 12 and 39 weeks (P < 0.001).

At 75 minutes post glucose and insulin infusion during the 2 week test, mean plasma insulin concentration was 1.31 µg/L (95% CI -0.81 - 3.44). This was not significantly different at 12 weeks (0.23 µg/L, 95% CI 0.18 - 0.28, P = 0.137), or at 39 weeks (0.45 µg/L, 95% CI 0.31 - 0.59, P = 0.191). Plasma insulin concentrations were different between 12 and 39 weeks (P = 0.002).

The AUCg, AUCn and AUCi did not differ between dietary groups at any of the test ages. There were no statistical differences between AUCg at 2 and 39 weeks. However at 12 weeks, the AUCg was significantly greater than at 2 weeks (P = 0.015) and at 39 weeks (P < 0.001, Table 5.6).

The AUCn and AUCi were not statistically different between 2 and 12 weeks and between 2 and 39 weeks. However, the AUCn was greater at 12 weeks than at 39 weeks (P = 0.016) and the AUCi was lower at 12 weeks than at 39 weeks (P < 0.001, Table 5.6).

The explanatory variable in the multivariable regression model for plasma glucose, plasma NEFA and plasma insulin concentration at 2, 12 and 39 weeks was time during the test, with an interaction with dietary group (full model output, Appendix D, Table D.3 - 11).

Table 5.5: Mean plasma glucose and NEFA concentrations throughout the CGIT test at 2, 12 and 39 weeks of age. There were no dietary group differences unless indicated (* = P < 0.05). Age related baseline plasma glucose and NEFA concentration differences are also denoted (** = P<0.05).

Time (mins)	Mean plasma glucose and NEFA concentrations (mmol/L, 95% CI)					
	Week 2		Week 12		Week 39	
	Glucose	NEFA	Glucose	NEFA	Glucose	NEFA
Baseline	5.61 (4.94 - 6.27)	0.61 (0.40 - 0.83)	5.11 (4.83 - 5.38)	1.09 (0.82 - 1.36)**	4.41 (4.06 - 4.75)**	0.70 (0.37 - 1.02)
1	9.26 (8.80 - 9.71)	0.74 (0.45 - 1.03)	10.66 (9.97 - 11.35)	0.95 (0.59 - 1.31)	12.14 (11.05 - 13.23)	0.67 (0.40 - 0.93)
5	8.00 (7.55 - 8.46)*	0.46 (0.24 - 0.69)	9.03 (8.77 - 9.30)	0.95 (0.80 - 1.11)	9.67 (9.19 - 10.15)	0.58 (0.40 - 0.75)
10	6.52 (6.00 - 7.03)	0.38 (-0.01 - 0.77)	7.66 (7.27 - 8.04)	0.60 (0.49 - 0.71)	7.73 (7.15 - 8.32)	0.42 (0.26 - 0.57)
15	5.55 (5.05 - 6.06)	0.24 (0.03 - 0.45)	6.81 (6.44 - 7.18)	0.45 (0.32 - 0.57)	6.43 (5.96 - 6.89)	0.32 (0.22 - 0.42)
25	3.93 (3.31 - 4.54)	0.22 (-0.03 - 0.46)	5.48 (5.10 - 5.87)	0.31 (0.21 - 0.41)	4.67 (4.13 - 5.21)	0.17 (0.12 - 0.22)
35	2.99 (2.32 - 3.66)	0.22 (0.07 - 0.36)	4.74 (4.35 - 5.14)	0.34 (0.26 - 0.42)	3.68 (3.04 - 4.31)	0.16 (0.12 - 0.20)
45	2.71 (2.00 - 3.42)	0.67 (0.35 - 0.98)	4.36 (3.95 - 4.77)	0.50 (0.40 - 0.60)	3.02 (2.41 - 3.62)	0.22 (0.15 - 0.29)
60	3.33 (2.53 - 4.13)	0.99 (0.57 - 1.40)	4.12 (3.75 - 4.48)	0.62 (0.45 - 0.78)	2.97 (2.47 - 3.46)	0.50 (0.38 -0.61)
75	3.72 (2.77 - 4.66)	0.16 (0.55 - 1.27)	4.21 (3.97 - 4.45)	0.71 (0.57 - 0.84)	3.45 (3.09 - 3.82)	0.60 (0.44 - 0.77)
90	3.97 (3.06 - 4.88)*	0.16 (0.53 - 1.23)	4.26 (4.04 - 4.49)	0.83 (0.59 - 1.08)	3.73 (3.39 - 4.06)	0.56 (0.38 - 0.74)
105	4.33 (3.37 - 5.29)*	0.85 (0.51 - 1.19)	4.43 (4.22 - 4.63)	0.89 (0.63 - 1.14)	3.92 (3.62 - 4.22)	0.65 (0.46 - 0.84)
120	4.56 (3.60 - 5.51)*	0.83 (0.34 - 1.31)	4.53 (4.29 - 4.77)	1.09 (0.90 - 1.28)	4.10 (3.84 - 4.35)*	0.66 (0.47 - 0.85)
135	4.90 (4.02 - 5.78)*	0.72 (0.41 - 1.04)	4.63 (4.37 - 4.89)	1.06 (0.77 - 1.34)	4.14 (3.91 - 4.37)*	0.67 (0.50 - 0.84)
150	5.10 (4.26 - 5.94)	0.77 (0.60 - 0.95)	4.64 (4.39 - 4.88)	1.00 (0.70 - 1.29)	4.25 (3.97 - 4.54)*	0.67 (0.52 - 0.83)

Table 5.6: Mean (95% CI) area under the curve for glucose (AUCg), NEFA (AUCn) and insulin (AUC_i) during the 3CGIT tests for animals in Group A and Group R.

Week of test	**AUCg (mmol/L/min)**		
	Group A	Group R	P value
2	338.4 (245.2 - 431.7)	284.6 (226.5 - 342.8)	0.119
12	372.0 (336.3 - 407.7)	363.2 (323.5 - 403.0)	0.341
39	278.1 (222.5 - 333.8)	288.0 (247.6 - 328.4)	0.360
Week of test	**AUCn (mmol/L/min)**		
	Group A	Group R	P value
2	49.5 (14.0 - 84.9)	63.6 (30.2 - 97.0)	0.236
12	58.7 (37.8 - 79.6)	61.4 (42.3 - 80.5)	0.406
39	39.0 (-2.9 - 81.0)	36.6 (25.3 - 48.0)	0.445
Week of test	**AUCi (µg/L/min)**		
	Group A	Group R	P value
2	89.8 (-59.8 - 239.5)	41.2 (24.3 - 58.1)	0.213
12	27.5 (19.6 - 35.3)	31.5 (20.8 - 42.1)	0.228
39	74.3 (45.6 - 103.1)	52.4 (30.2 - 74.6)	0.076

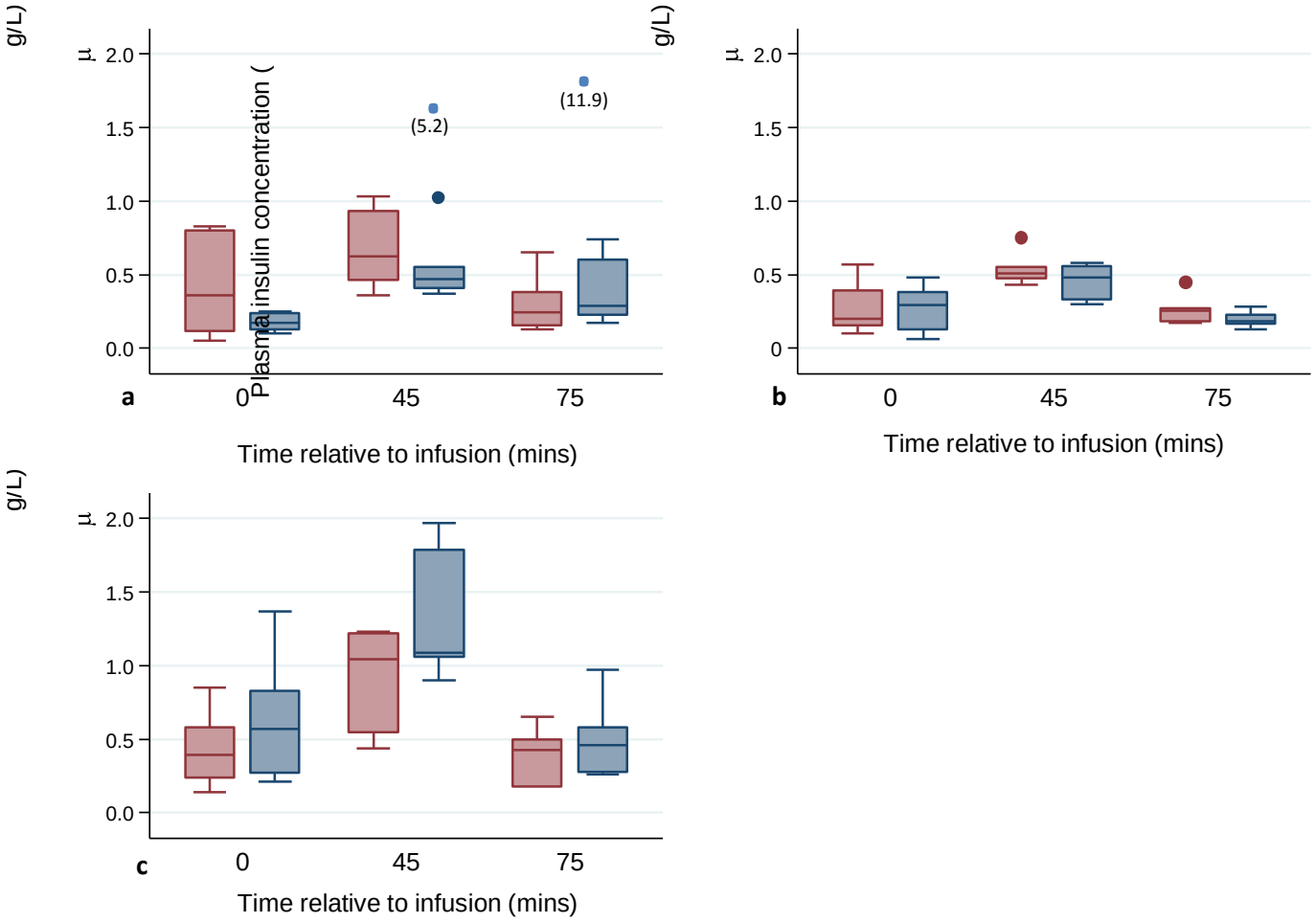

Figure 5.5: Box plots of mean plasma insulin concentrations at baseline (0 mins), 45 and 75 minutes after glucose and insulin infusion during the CGIT test for Group R (*n* = 6, red boxes) and Group A (*n* = 6, blue boxes) calves at a) 2, b) 12 and c) 39 weeks of age.

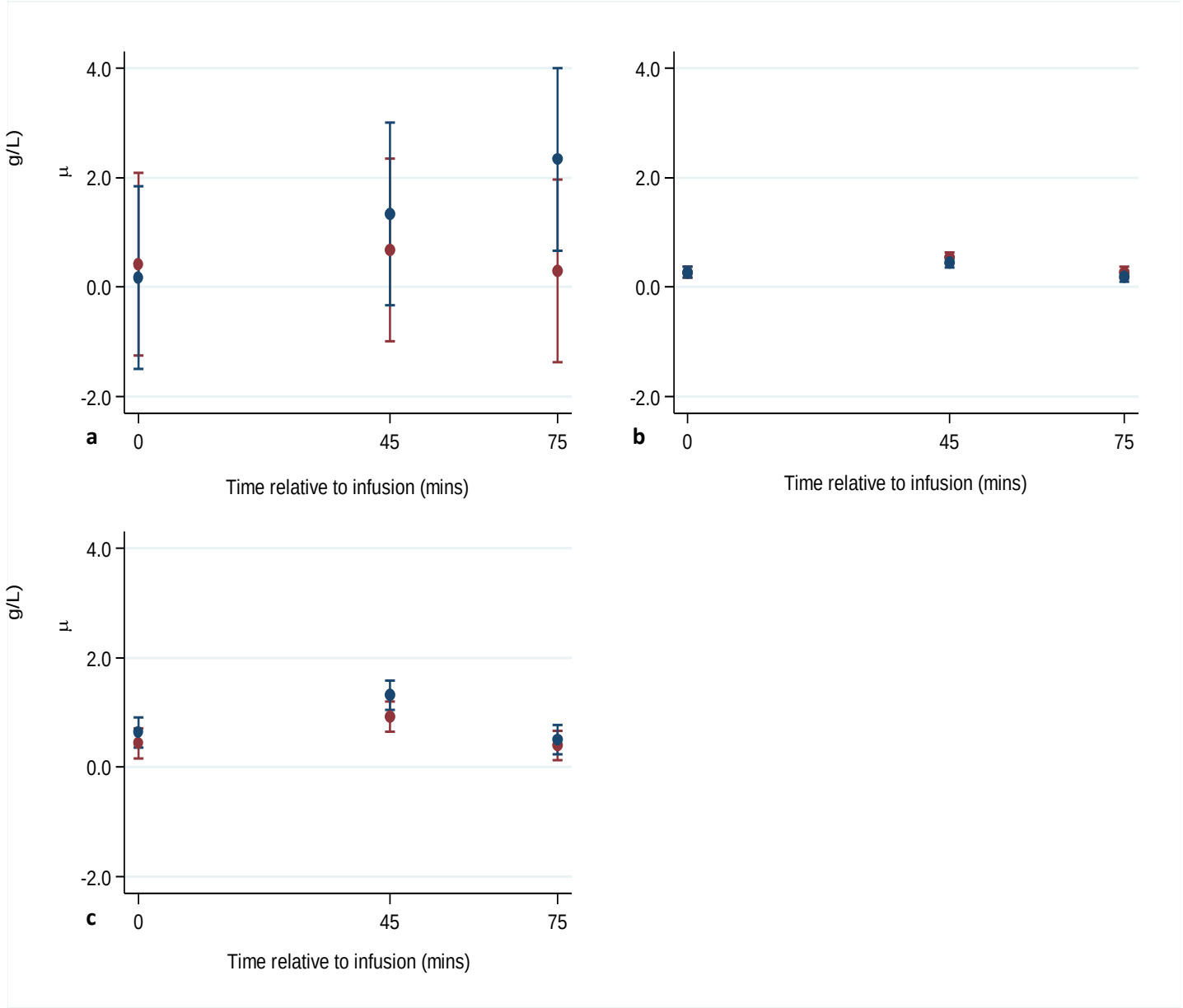

Figure 5.6: Marginal means (95% CI) of predicted plasma insulin concentration (µg/L) for calves in Group A (*n* = 6, blue line) and Group R (*n* = 6, red line) at 0, 45 and 75 minutes post glucose and insulin infusion during CGIT tests at a) 2 weeks, b) 12 weeks and c) 39 weeks.

5.4 Discussion

The primary aim of this study was to evaluate the CGIT test in Holstein calves as a method to identify failures or aberrations in glucose homeostasis. Previously, the GTT and IST have been used to assess glucose metabolism and insulin sensitivity in calves (Bach et al., 2013; Bossaert et al., 2009). However used independently, both of these tests have important limitations in that they can only respectively assess either the insulin secretion response or tissue sensitivity to insulin. The combined test simultaneously elicited responses to both exogenous glucose and insulin infusions that were largely comparable to those evidenced during the separate conduct of the GTT and IST in 2 week old animals. On the basis of the reproducibility of independently derived GTT and IST data within the combined test, the CGIT may offer a useful tool for the concurrent appraisal of the homeostatic response.

Animals were well handled from birth and displayed no behavioural stress responses. The tests were well tolerated by all the calves in the study and no clinical presentation of extreme hypoglycaemia was noted. That the CGIT test was well tolerated by calves is supported by the extensive literature describing the use of this test in the horse (Argo et al., 2012; Eiler et al., 2005; Johnson et al., 2011; McGowan et al., 2013).

The dose of glucose infused (150 mg/kg body weight) in this study was selected for use in both the GTT and CGIT following systemic evaluation of responses of large herbivores across studies which used both horses and calves (Bossaert et al., 2009; Bossaert et al., 2008; Boston et al., 2008; Eiler et al., 2005). This specific infusion dose has been demonstrated to avoid saturation of the cellular glucose transporters to minimise renal clearance (Bossaert et al., 2009; Eiler et al., 2005). Likewise, the exogenous insulin infusions (0.05 IU/kg body weight) used for the IST and CGIT tests were selected after consideration of published data (Bossaert et al., 2009).

Calves were uniformly fasted prior to testing on the basis that this would minimise metabolic differences between animals. Previous studies have limited fasting to a four hour period between feeding and GTT and IST onset in pre-ruminant calves (Bach et al., 2013; Bossaert et al., 2009). In light of the *ad libitum* MR diet of half of the calves in this study and the intent to re-evaluate animals after the onset of rumen function, it was considered that a 12 hour fast would be more appropriate. A 12 hour pre-test fast has been widely applied in

equine studies where caecal depletion after this time would be expected to be more complete than the rumenal clearance in animals during the 12 and 39 week tests of the current study (Eiler et al., 2005; McGowan et al., 2013). However, given that the CGIT test may have the potential for deployment on commercial farms, the adverse impacts following more prolonged fasts, especially if its use was later to be extended for application in lactating animals, was considered to be unacceptable in practice.

Baseline plasma glucose concentrations recorded for all tests during Study 1 were similar to those reported for neonatal dairy calves by other authors (Bossaert et al., 2009; Hostettler-Allen et al., 1994) and were significantly lower than concentrations recorded in insulin resistant veal calves (Hostettler-Allen et al., 1994). During Study 1, mean maximal plasma glucose concentrations following glucose infusions during the GTT and CGIT tests were similar (9.40 mmol/L), with return to baseline values at 52 and 19 minutes respectively. Comparative data from other studies for return to baseline times during the GTT in Holstein calves are up to 22 minutes less than recorded in the current study (Bach et al., 2013; Bossaert et al., 2009). Unmeasured variables such as genetic factors or length of pre-test fast could account for inter-study differences. In Study 1, insulin administration during the IST was associated with a mean decrease in plasma glucose concentration of 3.50 mmol/L, to 1.87 mmol/L by 35 minutes post insulin infusion, which was very similar to results during an IST in neonatal Holstein calves by Bossaert *et al* (1.78 mmol/L 30 minutes post insulin infusion). Following the hypoglycaemic nadir, only in 3 calves belonging to Group A did plasma glucose concentration increase back to original baseline values by the end of the test at 150 minutes. Although not statistically significant, this observation was interesting and may highlight extreme sensitivity to insulin of calves in Group R due to restricted feeding of MR and limited availability of glucose.

During the CGIT test in Study 1, glucose and insulin infusion elicited a biphasic glycaemic response. The mean plasma glucose concentration at the hypoglycaemic nadir was significantly greater during the CGIT test than during the IST (CGIT: 2.60 mmol/L, IST: 1.87 mmol/L, P = 0.030). However, only 3 calves from Group A were able to increase subsequent plasma glucose concentration to baseline values by the end of the CGIT test. On this basis, although less of a hypoglycaemic response was elicited during the CGIT test than during the IST, the insulin concentration infused during the combined test was considered to be

suitable for use in the Holstein dairy calf. A greater decrease in plasma glucose concentration would not have enabled return to baseline plasma glucose concentrations for any study animals. To ensure capture of the restoration of homeostatic control on blood glucose concentrations in future studies, the addition of a further sampling point, 180 minutes post-infusion should be considered.

The time taken to reach a hypoglycaemic nadir during the CGIT in Study 1 was significantly greater for calves in Group R than Group A (Group R: 65.0 minutes, Group A: 41.7 minutes, P = 0.020). This extended time may suggest prolonged endogenous insulin secretion in Group R calves, which may also explain why no Group R animals returned to baseline plasma glucose concentrations by the end of the IST.

It was of interest that application of the CGIT test alone, revealed possible differences in insulin/glucose dynamics between the nutritionally distinct calf groups although, statistically there were no differences in the baseline glucose concentrations, AUCg or time to return to baseline from the hyperglycaemic phase for any of the tests during Study 1.

During Study 2, the mean baseline plasma glucose concentration at 39 weeks was 4.41 mmol/L and was significantly lower than at 2 and 12 weeks ($P < 0.001$). Profound metabolic changes occur as calves develop from the pre-ruminant to the ruminant state. Neonatal calves consume a predominantly liquid diet, absorbing glucose directly through the small intestine. By contrast, the fully developed ruminant ferments ingested feedstuffs and assimilates the resultant volatile fatty acids (VFAs) as the primary metabolic substrate. As VFA synthesis increases with the maturation of ruminal function, blood glucose concentrations decrease. Thus, heifers with fully functional rumens (4.2 - 4.4 mmol/l (Brickell et al., 2009)) and adult Holstein cows (0.33 - 3.00 mmol/L, dependent upon stage of lactation (Bartley and Black, 1966; Bossaert et al., 2008)), could be predicted to have lower basal plasma glucose concentrations than pre-ruminant calves.

Baseline plasma insulin concentrations did not differ between pre-weaning dietary group for any of the 3 CGIT tests in Study 2. Mean plasma insulin values were greater at 39 weeks (0.54 µg/L) than at 2 (0.30 µg/L) and 12 weeks (0.27 µg/L). Regrettably, baseline insulin concentrations were not presented for the study described by Bach *et al (Bach et al., 2013),* where neonatal Holstein calves were challenged with a GTT. After conversion of units of

measurement of insulin concentration used by Bossaert *et al* (Bossaert et al., 2009) from µIU/mL to µg/L as described by Abuelo *et al* (Abuelo et al., 2012), baseline plasma insulin concentrations were determined at between 4 (weeks 2 and 12) and 7 (week 39) times greater in the current study than during Bossaert's study. The insulin kit used during the study described by Bossaert *et al* had been established in human medicine with only 25% cross-reactivity with bovine insulin. The insulin ELISA kit used for the current study was specifically developed for use with bovine samples (100% cross-reactivity), explaining the 4-fold greater plasma insulin concentration during the current study and a much more accurate representation of the true baseline plasma insulin concentration in 2 week Holstein calves.

Baseline NEFA concentrations throughout Study 2 were higher than that previously reported in young Holstein calves fasted for 4 hours (Bossaert et al., 2009; Stanley et al., 2002). This is a likely consequence of the imposition of a 12 hour pre-test fast in the current study. The mobilisation of NEFAs to maintain caloric homeostasis during periods of negative energy balance have been widely reported in adult Holstein cows, particularly around the time of parturition and onset of lactation (Bell, 1995; Overton and Waldron, 2004). An inverse relationship between plasma glucose and NEFA concentrations has previously been reported during periods of fasting in sheep and a comparable association was characterised for calves in the present study (Trenkle and Kuhlemeier, 1966). The fact that baseline plasma NEFA concentrations were greatest during the 12 week test (mean: 1.09 mmol/L compared to mean: 0.66 mmol/L at 2 and 39 weeks) suggested that increased lipolysis was occurring at and around the time of weaning for calves in both dietary groups (Bossaert et al., 2009). This may not be purely an effect of the 12 hour fast but may be also be associated with the transition from pre-ruminant to ruminant animal.

Despite a significantly lower mean baseline plasma glucose concentration at the 39 week CGIT test onset, mean maximal plasma glucose concentration at 1 minute post glucose and insulin infusion increased with age (week 2: 9.26 mmol/L, week 12: 10.66 mmol/L, week 39: 12.14 mmol/L). This probably reflected the age-related increase in the proportion of body weight occupied by the maturing ruminant digestive system, with a corresponding decrease in the relative somatic dilution pool, when glucose infusions were calibrated against total body weight.

The time to return to baseline plasma glucose concentration from the positive phase during the CGIT test in Study 2 was increased at 12 and 39 weeks compared to the 2 week test (36.7 minutes compared to 19.8 minutes, P <0.020). Although an increased time period of approximately 17 minutes was recorded between the 2 week and subsequent tests, values quoted for mature normo-glycaemic horses is similar (30 minutes (Eiler et al., 2005)) and for obese horses and ponies post weight loss is greater (84.6 minutes (Argo et al., 2012)). In addition, calves were exposed to only half the exogenous insulin dose used to drive glucose clearance in the equine studies. This would suggest that animals in the current study were relatively more insulin sensitive than mature equines.

Minimal plasma glucose concentrations during the negative phase of the CGIT test were greater at 12 weeks (3.93 mmol/L) compared to the 2 and 39 week tests (2.69 mmol/L). Visual appraisal of the glucose curve (Figure 5.4b) highlights the reduced magnitude of the negative glycaemic phase at 12 weeks compared to that at 2 and 39 weeks; this would be indicative of a less insulin sensitive population. This is further confirmed by the increased AUCg during the 12 week test compared to the 2 and 39 week tests. As there were no dietary group differences, this apparent reduction in efficiency of glucose metabolism and insulin sensitivity may be associated with weaning of these calves and may have a more prolonged effect than previously appreciated. Group A calves completed gradual weaning by 12 weeks of age whereas the Group R fed calves completed by 10 weeks. It is recognised that weaning is stressful for all animals regardless of the speed and method in which it is conducted and effects of this, such as reduced growth rates and increased disease incidence may be seen for weeks after (Bach et al., 2010; Quigley et al., 1991).

During GTT and CGIT tests, insulin sensitivity may be measured by calculating time taken to return to baseline plasma glucose concentration after glucose or glucose and insulin infusion, AUCg and AUCi. However, these measures may not present the full picture, as decreased insulin sensitivity may be compensated for by increased endogenous insulin production. The time required to return to baseline plasma glucose concentrations from the positive phase of the CGIT in Study 2 was similar between dietary groups, regardless of age. However, in order to fully ascertain if differences in insulin sensitivity were present in the current study, plasma insulin concentrations were recorded at 3 time points during Study 2 (McGowan et al., 2013).

The plasma insulin concentrations recorded at 45 minutes did not differ between 2 and 12 weeks or 2 and 39 weeks, but were greater at 39 weeks (1.12 μg/L) than at 12 weeks (0.50 μg/L). Similar differences were seen at 75 minutes, again with a greater value at 39 weeks (0.45 μg/L) than at 12 weeks (0.23 μg/L). Increased plasma insulin concentrations are often associated with insulin resistance, whereby a greater quantity of insulin is required to elicit a normal response to promote glucose homeostasis (Lebovitz, 2001).

During the 12 week CGIT test, reduced plasma insulin concentrations, a lower AUCi, increased baseline plasma NEFA concentrations (1.09 mmol/L, compared to 0.66 mmol/L at 2 and 39 weeks) and an increased AUCn was recorded. It was of concern that the above measures collectively indicated a decreased sensitivity to insulin and therefore a possible increase in the risk for metabolic disorders (Bossaert et al., 2009). However by the 39 week test, measures were similar to those recorded during the 2 week test of an insulin sensitive population. The implications of changes associated with weaning are worthy of further investigation.

To the author's knowledge, this is the first time that a combined test with the potential to evaluate glucose/insulin dynamics has been reported for Holstein dairy calves. The test appears robust and has potential for modification to allow application under practical field situations. Further work is required to explore its potential value as a juvenile predictor of adult health. Previous studies have suggested that feeding large volumes of milk or MR to dairy calves in discrete feeds may have a negative impact on insulin sensitivity (Bach et al., 2013; Hostettler-Allen et al., 1994). The major finding of this study is that the insulin sensitivity of calves fed *ad libitum* MR during early life appears unimpaired and efficiency of glucose metabolism was no different to that of calves fed restricted volumes of MR. The negative impact on insulin sensitivity due to consumption of large volumes of MR was not found when calves were managed *via* a true *ad libitum* MR feeding system. During the period of transition from pre-ruminant to ruminant animal, glucose metabolism may not be as efficient as during the pre-ruminant stage, although this reduction in efficiency was reversed when animals were 39 weeks of age.

5.5 References

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Chapter 6

Effect of milk replacer feeding strategy on the growth and productivity of Holstein heifer calves: from 12 weeks until pregnancy

6.1 Introduction

Dairy heifer rearing involves significant financial input and accounts for approximately 20% of total on farm costs (Dairy, 2011; Donovan et al., 1986; Gabler et al., 2000). Published data recommend that the optimal age at first calving (AFC) is 24 months (Ettema and Santos, 2004; Haworth et al., 2008; Keown and Everett, 1986). Below this age, heifers are unlikely to have sufficient body size to support their genetic potential for lifetime milk production or to easily deliver a healthy calf (Ettema and Santos, 2004). Conversely, rearing costs will be increased for animals with a greater AFC (Brickell et al., 2009a). Well grown, healthy heifers that are able to calve for the first time at 24 months should therefore be the goal for all dairy farmers with Holstein herds. Although it is widely known that achieving this optimal AFC depends largely on early life management and nutrition (Brickell et al., 2009a; Morrison et al., 2012; Waltner-Toews et al., 1986; Wathes et al., 2008), farmers often miss this opportunity to maximise the production potential of their replacement animals by implementing sub-optimal rearing strategies (Brickell et al., 2009a; Cole and Null, 2010).

More recently, research into so called 'accelerated' or 'enhanced' pre-weaning feeding strategies has highlighted the ability of dairy calves to grow at rates of close to 1kg per day during the milk fed period (Appleby et al., 2001; Hill et al., 2013; Jasper and Weary, 2002; Van Amburgh et al., 1998). Similar growth rates can be maintained throughout the pre-pubertal post-weaned period, attainment of this goal will ensure well grown heifers that are ready to enter the productive stage of life by 24 months (Zanton and Heinrichs, 2007). It has been contested that high growth rates in Holstein heifers may have negative implications associated with increased deposition of fat and consequent impacts on udder development (Lammers et al., 1999; VandeHaar, 2001). By contrast, other studies have found that there is an increase of between 32 and 47% in mammary DNA content of calves fed twice as much milk replacer throughout the pre-weaning phase (1kg vs. 0.5kg per day) (Brown et al., 2005a; Meyer et al., 2006; Sejrsen, 1994), suggesting enhanced somatic growth is accompanied by mammary tissue hyperplasia. Sejrsen *et al* (Sejrsen, 1994) found no negative effect on mammary development (measured by determination of mammary parenchyma tissue) when pre-weaned calves were given *ad libitum* access to milk replacer. In addition, studies which assessed whole body composition of calves fed at higher planes of

nutrition, failed to provide evidence that this was associated with increased fat deposition (Bartlett et al., 2006).

For a heifer to calve for the first time at the optimal age she should be served by 13 - 14 months of life; however age should not be used as the sole determinant of when to serve. Sufficient growth prior to service must be achieved to enable the establishment and support of a pregnancy to term and the ability to deliver a calf with relative ease (Wathes et al., 2014).

On farm management strategies to determine the timing of a heifer's first service varies greatly between individual enterprises but is one of the most important decisions impacting on an animal's lifetime productivity and profitability (Dairy Co., 2011). Although it is generally agreed that an age of 24 months at first calving is financially optimal (Keown and Everett, 1986), there is considerable debate as to the ideal weight and height targets for first service in Holstein heifers, with a paucity of peer reviewed recommendations. Part of the problem is that the Holstein breed has changed remarkably in terms of mature body size over the past 10-20 years, with animals becoming heavier and taller in stature (Bermingham et al., 2006).

Post weaned heifers should be provided with a diet that allows growth at a sufficient rate without fattening (Drackley, 2008). When groups of dairy heifers (4.5 - 9.5 months) were fed either a standard (700g/day weight gain) or accelerated (1000g/day weight gain) feeding regime, it was determined that the animals given the higher plane of nutrition reached puberty at least one month earlier than the animals fed on the standard diet [Lammers et al., 1999]. These data emphasise that, in order to benefit from this earlier achievement of puberty, heifers should be served at an appropriate size and weight, as opposed to waiting and serving them at an age appropriate for less well grown heifers. For farmers to capitalise on the advantage gained from good growth of heifers, it is essential to define clear height and weight targets in combination with good heat detection for animals that are eligible for service.

The somatotropic axis, consisting of growth hormone (GH), insulin like growth factors I and II (IGF-1 & IGF-2) and their associated proteins and receptors, plays an important role in controlling physiological and metabolic processes within all mammalian species (Renaville et

al., 2002). Complex feedback mechanisms facilitate GH and IGF-1 availability. Stimulated by GH, IGF-1 is secreted predominantly by the liver. Circulating concentrations of IGF-1 are strongly influenced by nutrition and have been found to be associated with growth (especially skeletal muscle, cartilage and bone), development and fertility in cattle (Bartlett et al., 2006; Kinsbergen et al., 1994).

In addition to immunoglobulins, colostrum contains many other components including growth factors and hormones (Cortese, 2009; Godden, 2008; Kelly, 2003). However, colostral IGF-1 is not absorbed by the neonatal calf in vast quantities (Hammom and Blum, 1997), but the concentration of IGF-1 in the calf's circulation is affected by the amount of colostrum consumed (Grutter and Blum, 1991). This implies that for newborn calves (<48 hours), circulating IGF-1 is derived from endogenous origins (Hammom and Blum, 1997). Reductions in the amount of or delay in consumption of colostrum has been associated with a reduction of subsequent circulating IGF-1 concentration in young calves (Hammon et al., 2000), highlighting that correct colostrum feeding of new born calves is not just important for immediate immunoglobulin absorption. Following on from this, calves fed high protein and energy diets during the first 3 months of life had increased growth rates with correspondingly increased plasma IGF-1 concentration (Brown et al., 2005b), confirming that IGF-1 is associated with nutritional status. Circulating IGF-1 concentration has therefore been suggested as an indicator of growth (Brickell et al., 2009b; Radcliff et al., 2004). It is also well documented that circulating concentrations of IGF-1 are elevated around the time of puberty onset in Holstein heifers (Gluckman et al., 1987; Velazquez et al., 2008), and can therefore be used as a tool to confirm timing of puberty.

This study assessed the IGF-1 concentrations and post-weaned growth of a cohort of Holstein heifers that had been enrolled onto a dietary intervention study from birth until 12 weeks of age. Particular attention was paid to the age at which these animals reached puberty, first service and when first pregnancy was achieved.

6.2 Materials and Methods

The study was performed between April 2011 and April 2014 at the University of Liverpool's Wood Park Dairy Farm, Neston, Wirral, U.K. (53°N). The farm milked approximately 170 Holstein-Friesian cows with an annual lactation yield of around 10,500 litres on a 3 times daily milking regime. All adult cows were housed year round apart from during the last 100 days of lactation during which they were allowed access to pasture during the summer months. All non-lactating, pregnant (dry) cows were housed throughout the 8 week dry period. The calving pattern on the farm was year round with no seasonal trends.

Study heifers were recruited at birth and entered into either an *ad libitum,* Group A (*n* = 50) or restricted, Group R (*n* = 50) MR feeding strategy for the first 12 weeks of life (Thesis Chapter 3).

From 12 weeks of age onwards, calves in both dietary groups were subject to common nutritional and husbandry protocols. Irrespective of pre-weaning dietary group, at 4 months of age all calves were transferred to follow-on accommodation (indoor straw yards 18m x 6m, approximately *n* = 12 per group). From 3 to 5 months of age, the diet consisted of a maximum of 2.5kg of concentrate feed per head (86.20% DM, 18.00% crude protein, 4.00% oil, 9.50% ash, 12.50% crude fibre , ME 14.551 MJ/Kg Super Rearer 18 nuts, BOCM Pauls, U.K.). Grass hay and fresh water was available at all times.

In accordance with standard farm practice, nutrition from 5 months onwards was highly variable consisting largely of refusals from the total mixed rations fed to the lactating, far off dry and transition dry cows. However, depending on the quantity of refusals available each day, additional maize silage or grass silage was added to the diet.

Measurements

All procedures were performed under the U.K. Animals (Scientific Procedures) Act, 1986. The body weight, withers and loin height, heart and belly girth, CRL, HFL and BCS of each heifer was recorded as previously described (Thesis Chapter 3) every 4 weeks from 12 weeks of age until attainment of pregnancy.

In addition, blood samples were taken as previously described (Chapter 3) on a weekly basis from 28 weeks of age until the onset of puberty to harvest plasma. Progesterone was analysed as 'pregnane metabolites' using an established plasma ELISA which had been previously validated for use in cattle (Walker et al., 2008). The minimum detectable concentration was 0.08 ng/ml, intra and inter-assay variation were 8.3 and 14% respectively. Animals were classified as pubertal when plasma pregnane metabolite concentrations of ≥2.00 ng/ml were recorded for 2 consecutive weeks.

Further blood samples were collected (as described in Thesis Chapter 3) from half of the study animals, chosen at random (Group A, *n* = 25, Group R, *n* = 25) to assess plasma IGF-1 concentration at key times across both the pre and post weaned periods. Samples analysed were taken from animals at 48 hours, 3 weeks, 20 weeks and when animals reached 400kg body weight (mean age 57.7 weeks, 95% CI 56.6 - 58.8). IGF-1 concentration was determined in duplicate using a two site immunoenzymometric assay (IGF-1 Direct ELISA kit, Oxford Biosystems Cadama, U.K.). Assay sensitivity was 3.1 µg/L and inter and intra-assay coefficients of variation were 5.5% and 5.5% respectively.

A body weight of 380 kg and withers height of 125 cm were set as minimum standards which animals must have to achieve before first service in the present study. All heifers were served *via* artificial insemination of selected semen following once per day visual heat detection (no synchronisation or other heat detection methods were employed). Body weight and withers height at service together with age at puberty, age at first service and age at conception are referred to as key performance indicators (KPI's) in the context of this study.

Statistical Analyses

All data were initially entered into an Excel spreadsheet (Microsoft Corp, U.S.A.) and exported to STATA 13 (StataCorp, Texas, U.S.A.) for analysis.

Body weight and Morphometric measures: Daily changes of body weight and all morphometric measures from 12- 108 weeks of age were calculated for discrete 4 week time periods throughout the study. Students t tests were used to compare the mean measurements at different time points between calves in Group A and Group R.

Following simple univariable regression, a random effects multivariable regression model was fitted with body weight as the outcome variable. A backward stepwise process was employed for selection of final explanatory variables. Variables were retained in the model if they improved model fit as assessed by likelihood ratio testing ($P < 0.200$). The final explanatory variables for the body weight model were forced into random effects regression models for the other morphometric measures (withers and loin height, heart and belly girth, CRL, HFL and BCS).

The following variables were initially offered to the full body weight model: pre-weaning dietary group (*ad libitum* or restricted MR), age in weeks, an age*diet interaction term, dam parity, plasma TP and occurrence of diarrhoea and/or pneumonia during the first 12 weeks. Calf and bull identity were included as a random effects. In all models, the full dataset (from birth till conception) was used.

Plasma IGF-1: Initially, simple univariable analysis using Students t tests and simple linear regression was carried out to investigate the association between mean IGF-1 concentration and dietary group (restricted or *ad libitum* MR) at all four sample time points.

Following univariable analysis, a multivariable regression model was fitted with IGF-1 concentration as the outcome variable. Calf identity was included as a random effect. A backwards stepwise selection procedure was employed for selection of the final explanatory variables using likelihood ratio testing; variables with a P value of <0.050 remained in the model.

The following explanatory variables were offered to the initial model: dietary group (restricted or *ad libitum* MR) with an interaction with age in weeks, body condition score and body weight with calf identity as a random effect.

The average daily weight gain (ADG) of heifers from birth to 3 weeks, 3 to 20 weeks, 20 to 58 weeks and overall (0 - 58 weeks) was calculated. Assessment of association between ADG and IGF-1 concentration at all time points was carried out *via* linear regression analysis.

For all weight, morphometric and plasma IGF-1 analyses, marginal means (95% CI) adjusted for confounders were estimated from multivariable regression models and are presented where appropriate.

Survival Analyses: Survival analysis was employed to assess the impact of pre-weaning diet on the age at puberty onset, first service, age at conception, age at attaining 125cm in withers height and age at attaining 380kg in body weight. Kaplan Meier survival curves were plotted for group A and R separately. Survival curves were compared using the Log-rank (lr) test. Cox proportional hazard models were fitted for all outcomes to assess the hazard ratio (relative risk) for potential explanatory variables. Proportional hazard assumptions for Cox's regression were checked using Schoenfeld residuals and were accepted if $P > 0.050$.

Following survival analysis of data for age at puberty onset, age at first service and age at attainment of pregnancy, further more specific analysis was carried out. For each dataset separately, the upper and lower quartiles of animals to reach the event of interest were extracted from the original data to form new datasets. The impact that birth weight, plasma TP, pre-weaning dietary group, pre-weaning disease and BCS at 3 weeks of age had on time to reach these key targets (puberty, first service and pregnancy) were assessed using Student's t tests (continuous data) and chi squared tests (non-continuous data).

To explore the relative contribution of the afore-mentioned explanatory variables on time to first service for the whole cohort of heifers, a multivariable Cox regression model was fitted. Variables initially offered to the model were; pre-weaning dietary group, birth weight, plasma TP, incidence of diarrhoea during the first 12 weeks and incidence of pneumonia during the first 12 weeks. A backward stepwise process was employed for

selection of final explanatory variables. Variables were retained in the model if they improved model fit as assessed by likelihood ratio testing ($P < 0.200$).

6.3 Results

Of the 100 calves which entered the study, 98 animals remained within the cohort at the end of the study period (68.9 weeks, 95% CI 66.6 - 71.3). Two animals, one from each dietary group, died prior to reaching eligibility for first service (accidental death), these animals were excluded from further analyses. Data were analysed with respect to body weight, morphometric measures, key performance indicators and plasma IGF-1 concentration.

Body weight

Univariable analyses: Mean average daily weight gain from 12 to 60 weeks (Group R, *n* = 49; Group A, *n* = 49) was 0.837 kg daily (95% CI, 0.815 - 0.860) with no dietary group differences (P = 0.771). The mean daily body weight changes during every 4 week period throughout the entire post weaning period are presented in Table 6.1.

Calves in Group A had significantly higher average daily weight gains at 12-16 weeks and 60-64 weeks than calves in Group R; during all other time periods there were no significant differences in average daily weight gain between dietary groups (Table 6.1).

Dietary group, dam parity, birth weight, diarrhoea during the pre-weaning period and plasma TP were all positively associated with body weight whilst, pneumonia during the pre-weaning period was negatively associated (Table 6.2).

Multivariable analyses: Explanatory variables that remained in the final bodyweight model were: pre-weaning dietary group (*ad libitum* or restricted MR) with an interaction with age in weeks, dam parity, plasma TP, the presence of diarrhoea in the first 12 weeks and presence of pneumonia in the first 12 weeks (Table 6.3).

The impact of dietary group on body weight was most marked during the first 3 - 4 weeks of life (Figure 6.1 & Chapter 3). Thereafter, the rate of change of weight was similar for both dietary groups as visually appreciable by parallel slopes (Figure 6.1a).

Morphometric measures

Univariable analyses: At the onset of the post-weaned period (12 weeks of age), all morphometric measures for calves in the Group A were greater than those recorded for calves in Group R with the exception of belly girth. However, over the course of the post weaning period, the differences in morphometric measures between dietary groups progressively decreased and became numerically undetectable. The ages at which these differences were lost differed between measures were: withers and loin height, 26 weeks; heart girth, 72 weeks; HFL, 52 weeks; CRL, 56 weeks and BCS 16 weeks (Appendix E, Tables E.1 - E.7). Belly girth was significantly greater in Group A calves from week 16 to 72 after which, group differences were lost.

Dietary group, birth weight and plasma TP were all positively associated ($P < 0.05$) with morphometric measurements (Table 6.4). Associations between morphometric measures and the other potential explanatory variables varied both in direction and significance.

Multivariable Analyses: Explanatory variables offered to all models were identical to those found previously to be significant explanatory variables from the body weight analysis, (i.e. pre-weaning dietary group [*ad libitum* or restricted MR] with an interaction with age in weeks, dam parity, plasma TP, diarrhoea during the first 12 weeks and pneumonia during the first 12 weeks).

Visual appraisal of the predicted changes in morphometric measures suggested that the rates of increase in heart girth (Figure 6.1c), withers height (Figure 6.2a), loin height (Figure 6.2b), CRL (Figure 6.2c) and HFL (Figure 6.2d) were progressively decreasing as the study progressed. Belly girth values differed from others in that recorded measures for each group had different slopes, one with a more rapid rate (approximately 3.3 cm/week) of increase from 12 to 24 weeks, then a slower rate (approximately 1.1 cm/wk) of increase from 24 weeks onwards (Figure 6.1d). Average daily changes over each 4 week period from 12 weeks of age onwards for each morphometric measure are presented in Appendix E, Tables E.1-E.7.

For all morphometric measures overall, Group A calves had higher recorded values than Group R calves but this difference was not statistically significant at all time points. There

was a trend for pre-weaning diet-associated differences to decrease over time, such that by the end of the study there were no differences attributed to pre-weaning dietary group in any of the morphometric measures with the exception of body weight.

Although mean BCS remained higher in Group A than Group R animals throughout the study period, there was a trend for BCS to increase in both dietary groups from approximately 36 weeks of age onwards (Figure 6.1b).

Table 6.1: Mean average daily weight gains for calves in both Group A and R at 4 week intervals throughout the post-weaning period.

Age (weeks)	**Mean average daily weight gain (kg) 95% CI**		**P Value**
	Group A (95% CI, *n*)	**Group R (95% CI, *n*)**	
12.00 - 15.99	1.134 (1.074 - 1.194, 50)	1.015 (0.949 - 1.081, 50)	0.009
16.00 - 19.99	0.842 (0.730 - 0.954, 50)	0.932 (0.859 - 1.006, 50)	0.179
20.00 - 23.99	0.911 (0.807 - 1.014, 49)	0.811 (0.725 - 0.898, 50)	0.141
24.00 - 27.99	0.687 (0.583 - 0.791, 49)	0.733 (0.628 - 0.838, 50)	0.531
28.00 - 31.99	0.646 (0.539 - 0.753, 49)	0.677 (0.472 - 0.877, 50)	0.788
32.00 - 35.99	0.862 (0.750 - 0.973, 49)	0.764 (0.662 - 0.868, 50)	0.203
36.00 - 39.99	0.901 (0.818 - 0.984, 49)	0.926 (0.813 - 1.040, 50)	0.360
40.00 - 43.99	0.984 (0.860 - 1.108, 49)	0.913 (0.804 -1.022, 50)	0.390
44.00 - 47.99	0.966 (0.833 - 1.099, 49)	0.981 (0.888 - 1.074, 49)	0.850
48.00 - 51.99	0.719 (0.565 - 0.872, 49)	0.828 (0.731 - 0.925, 49)	0.235
52.00 - 55.99	0.867 (0.701 - 1.034, 49)	0.816 (0.662 - 0.969, 49)	0.647
56.00 - 59.99	0.577 (0.433 - 0.720, 49)	0.680 (0.563 - 0.797, 49)	0.265
60.00 - 63.99	0.989 (0.847 - 1.132, 47)	0.773 (0.637 - 0.908, 46)	0.029
64.99 - 67.99	0.761 (0.580 - 0.941, 37)	0.909 (0.751 - 1.067, 38)	0.107
68.00 - 71.99	0.878 (0.709 - 1.047, 24)	0.681 (0.414 - 0.948, 28)	0.224
72.00 - 75.99	0.768 (0.333 - 1.203, 12)	0.744 (0.539 - 0.949, 18)	0.906
76.00 - 79.99	0.746 (0.183 - 1.301, 8)	0.835 (0.557 - 1.114, 13)	0.720
80.00 - 83.99	1.125 (0.363 - 1.887, 4)	0.938 (0.593 - 1.282, 8)	0.496
84.00 - 87.99	0.714 (1)	0.013 (0.241 - 0.985, 6)	
88.00 - 91.99	0.357 (1)	1.071 (0.646 -1.497, 4)	
92.00 - 95.99	0.286 (1)	0.667 (0.564 - 0.759, 3)	
96.00 -99.99	1.071 (1)	1.429 (-6.740 - 9.597, 2)	
100.00 - 103.99	0.286 (1)	0.000 (-2.723 - 2.723, 2)	
104.00 - 107.99	0.643 (1)	0.500 (1)	

Table 6.2: Individual regression analyses to assess variables that may be associated with body weight. The regression equations included age (in weeks) in addition to the variable in question, but were unadjusted for other variables. Full models are presented in Appendix E, Table E.8.

Outcome variable: Body weight			
	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	1.545	0.608 - 2.481	0.001
Diarrhoea (first 12 weeks)	6.457	2.909 - 10.004	<0.001
Pneumonia (first 12 weeks)	-4.700	-8.343 - -1.057	0.011
Plasma TP	6.714	4.544 - 8.883	<0.001
Birth weight	2.484	2.194 - 2.773	<0.001
Dietary group (*ad libitum* or restricted MR)	17.797	14.376 - 21.218	<0.001

Table 6.3: Multivariable regression model for the association between body weight and dietary group, including potential confounders. Calf was included as a random effect. The residual variance attributed to individual calf ID was 34.3% (95% CI 28.0 - 41.4). Coefficients for time (in weeks) and interaction terms are omitted for clarity (full model is presented in Appendix E, Table E.9).

Outcome variable: Body weight	**Coefficient**	**95 % CI**	**P value**
Dietary group (*ad libitum* or restricted MR)	5.740	-0.842 - 12.322	0.087
Dam parity (heifer vs. cow)	7.692	2.448 - 12.936	0.004
Plasma TP	3.626	0.391 - 6.860	0.028
Pneumonia (first 12 weeks)	-8.431	-14.616 - -2.246	0.008
Diarrhoea (first 12 weeks)	-4.521	-10.184 - 1.142	0.118
Constant	15.958	-6.610 - 38.526	0.166

Random-effects Parameters	Estimate	95% CI
calf:	157.160	117.024 - 211.062
Residual	300.417	287.161 - 314.286

Table 6.4: Regression analyses to assess variables that may have impacted on morphometric measures. The regression equation included age (weeks) in addition to the variable in question but results were unadjusted for other variables.

Outcome variable: Withers Height	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	-0.063	-0.199 - 0.074	0.367
Diarrhoea (first 12 weeks)	-0.114	-0.631 - 0.404	0.667
Pneumonia (first 12 weeks)	0.306	-0.224 - 0.837	0.258
Plasma TP	0.630	0.313 - 0.947	<0.001
Birth weight	0.332	0.289 - 0.374	<0.001
dietary group (*ad libitum* or restricted MR)	1.002	0.491 - 1.513	<0.001
Outcome variable: Loin height	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	0.041	-0.096 - 0.179	0.554
Diarrhoea (first 12 weeks)	-0.397	-0.916 - 0.123	0.135
Pneumonia (first 12 weeks)	0.014	-0.519 - 0.547	0.959
Plasma TP	0.668	0.350 - 0.987	<0.001
Birth weight	0.327	0.284 - 0.369	<0.001
dietary group (*ad libitum* or restricted MR)	1.318	0.807 - 1.830	<0.001
Outcome variable: Heart girth	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	0.371	0.167 - 0.573	<0.001
Diarrhoea (first 12 weeks)	1.206	0.434 - 1.979	0.002
Pneumonia (first 12 weeks)	-0.020	-0.813 - 0.774	0.961
Plasma TP	0.703	0.222 - 1.183	0.004
Birth weight	0.463	0.399 - 0.528	<0.001
dietary group (*ad libitum* or restricted MR)	3.792	3.047 - 4.537	<0.001
Outcome variable: Belly girth	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	0.518	0.221 - 0.816	0.001
Diarrhoea (first 12 weeks)	0.619	-0.510 - 1.747	0.282
Pneumonia (first 12 weeks)	0.019	-1.139 - 1.176	0.975
Plasma TP	1.492	0.794 - 2.191	<0.001
Birth weight	0.614	0.519 - 0.708	<0.001
dietary group (*ad libitum* or restricted MR)	3.875	2.772 - 4.978	<0.001
Outcome variable: Hock-fetlock length	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	0.015	-0.041 - 0.071	0.595
Diarrhoea (first 12 weeks)	-0.191	-0.402 - 0.020	0.075
Pneumonia (first 12 weeks)	0.249	0.033 - 0.465	0.024
Plasma TP	0.279	0.149 - 0.409	<0.001
Birth weight	0.110	0.093 - 0.128	<0.001
dietary group (*ad libitum* or restricted MR)	0.378	0.170 - 0.587	<0.001
Outcome variable: Crown rump length	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	0.122	-0.121 - 0.365	0.324
Diarrhoea (first 12 weeks)	-0.139	-1.059 - 0.781	0.767
Pneumonia (first 12 weeks)	0.242	-0.701 - 1.185	0.615
Plasma TP	0.846	0.279 - 1.413	0.003
Birth weight	0.485	0.408 - 0.563	<0.001
dietary group (*ad libitum* or restricted MR)	3.837	2.945 - 4.730	<0.001
Outcome variable: Body condition score	Coefficient	95% CI	P Value
Dam parity (heifer vs cow)	0.012	0.004 - 0.020	0.004
Diarrhoea (first 12 weeks)	0.123	0.094 - 0.153	<0.001
Pneumonia (first 12 weeks)	-0.107	-0.138 - -0.076	<0.001
Plasma TP	0.063	0.045 - 0.082	<0.001
Birth weight	0.008	0.006 - 0.011	<0.001
dietary group (*ad libitum* or restricted MR)	0.072	0.042 - 0.102	<0.001

Table 6.5: Coefficients (95% CI) for all remaining explanatory variables in the 7 morphometric multivariable models. Coefficients for dietary group and interaction terms are not presented, *denotes P < 0.200, **denotes P < 0.05 (Appendix E, models E.10-E.15 for full output).

Outcome variable / **Explanatory variables**	withers height	loin height	heart girth	belly girth	crown to rump length	hock-fetlock length	body condition score
plasma total protein	0.612* (-0.139 - 1.362)	0.756** (0.071 - 1.441)	0.681* (-0.255 - 1.616)	1.342** (0.112 - 2.572)	0.837* (-0.250 - 1.924)	0.267** (0.032 - 0.503)	0.052** (0.013 - 0.091)
dam parity	0.773 (-0.444 - 1.991)	0.827 * (-0.284 - 1.937)	3.099** (1.582 - 4.617)	4.348** (2.353 - 6.344)	1.573** (-0.190 - 3.336)	0.355* (-0.026 - 0.737)	0.024 (-0.040 - 0.087)
pneumonia (1^{st} 12 weeks)	-0.691 (-2.126 - 0.745)	-1.311** (-2.621 - 0.001)	-2.065** (-3.855 - 0.275)	-2.597** (-4.951 - 0.244)	-2.157** (-4.237 - -0.078)	-0.035 (-0.485 - 0.415)	-0.074* (-0.148 - 0.001)
diarrhoea (1^{st} 12 weeks)	-0.810 (-2.124 - 0.505)	-1.290** (-2.489 - -0.091)	-1.055 (-2.694 - 0.585)	-2.182** (-4.338 - 0.027)	-2.463** (-4.367 - 0.558)	-0.478** (-0.890 - -0.065)	0.010 (-0.058 - 0.079)

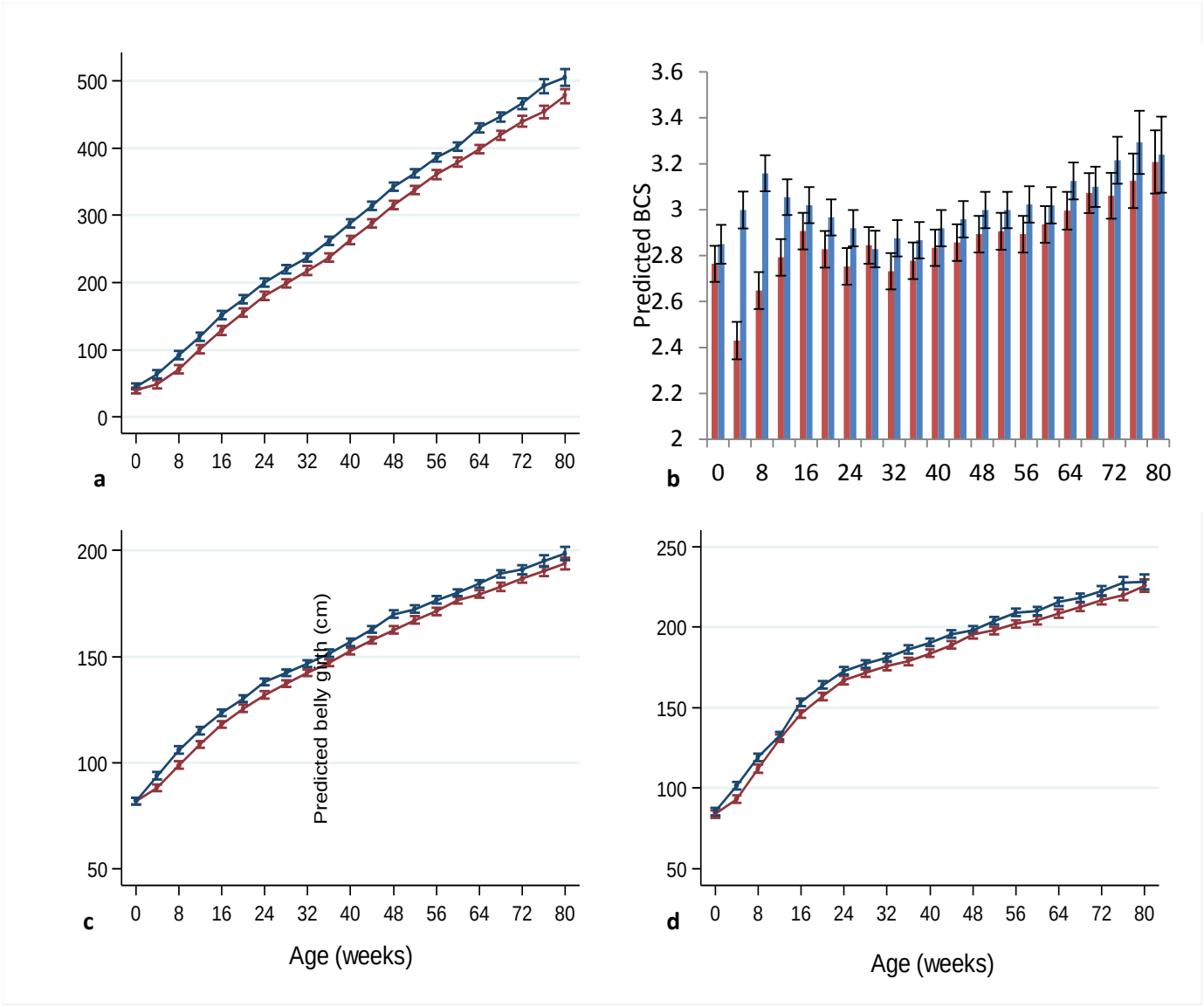

Figure 6.1: Marginal means (95% CI) of a) predicted body weight (kg), b) predicted BCS, c) predicted heart girth (cm) and d) predicted belly girth (cm) for calves in Group A (blue line) and R (red line) from birth until 80 weeks of age.

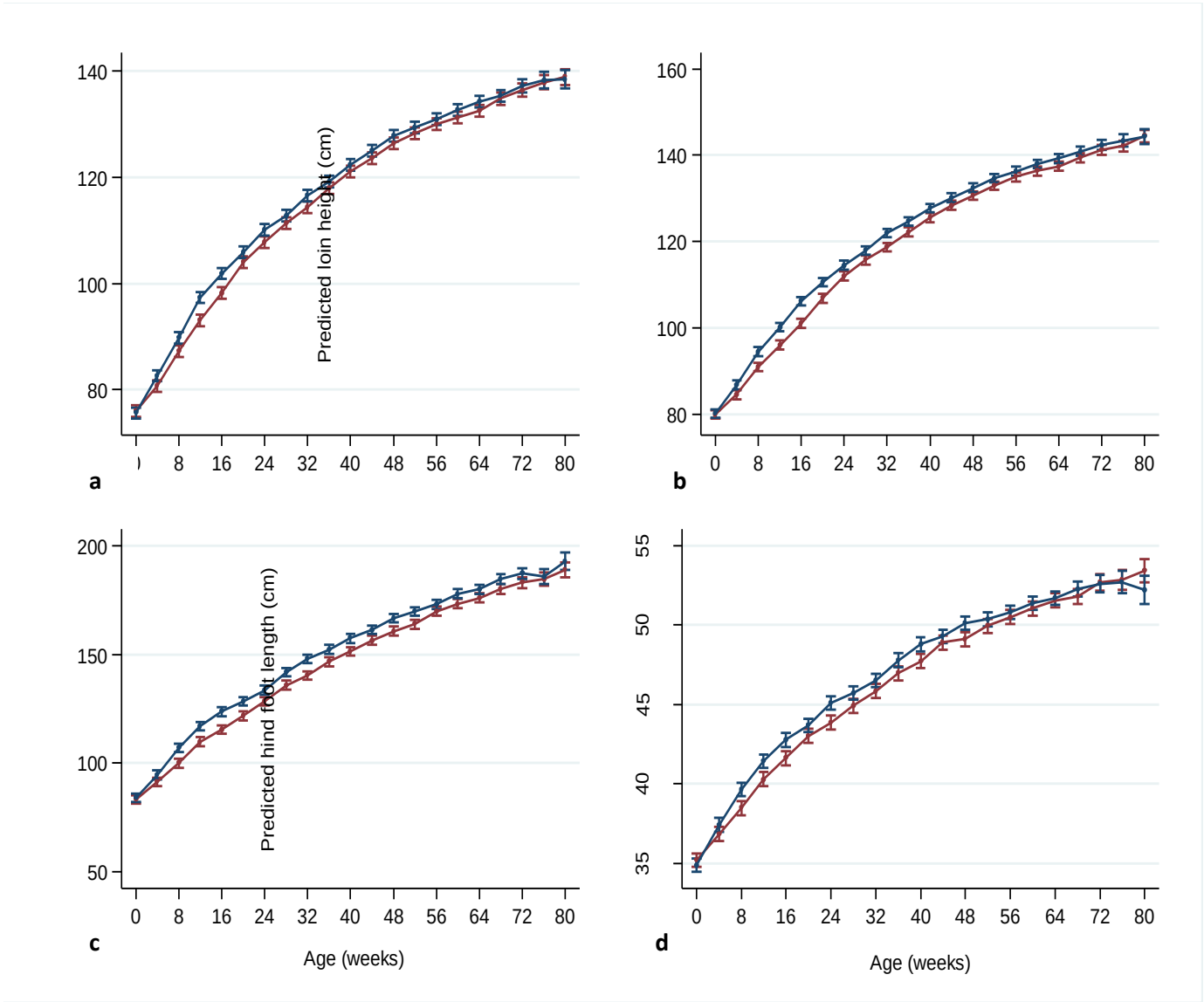

Figure 6.2: Marginal means (95% CI) of a) predicted withers height (cm), b) predicted loin height (cm), c) predicted crown rump length (cm) and d) predicted hock-fetlock length (cm) for calves in Group A (blue line) and R (red line) from birth until 80 weeks of age.

Puberty & Conception Data

Univariable Analyses: Calves from Group A reached measured KPI's more quickly than Group R animals. Group A reached puberty 2.2 weeks earlier, first service 2.6 week earlier and conceived for the first time 2.3 weeks earlier than Group R heifers.

The mean age at the onset of puberty was 41.6 (95% CI 39.2 - 44.1) weeks for Group A calves and 43.8 (95% CI 41.6 - 46.0) weeks for Group R calves (P = 0.096). The age at puberty onset was not affected by disease (diarrhoea or pneumonia) during the pre-weaning period (P = 0.469). The mean age at first service was 62.7 weeks (95% CI 61.5 - 63.9) for Group A and 65.3 (95% CI 62.6 - 68.0) weeks for Group R (P = 0.038) and the age at conception was 67.8 (95% CI 64.7 - 71.0) weeks for Group A and 70.1 (95% CI 66.6 - 73.6) weeks for Group R (P = 0.171). Neither age at first service or conception were affected by disease during the pre-weaning period. Forty nine animals in Group A and 46 animals in Group R conceived.

The mean number of services required to achieve conception was similar between dietary groups (2.02; 95% CI 1.66 - 2.38, P = 0.998). Fifty seven percent of group A and 51% of Group R animals became pregnant after the first service (P = 0.550).

There were no dietary group differences in body weight, withers height or BCS at the onset of puberty, first service and conception (P > 0.05) (Table 6.6).

Based on a 285 day gestation period, predicted calving ages for heifers from Group A was 25.3 months (95% CI 24.6 - 26.1), and Group R animals was 25.9 months (95% CI 25.0 - 26.7) (P = 0.171).

Table 6.6: Mean (95% CI) weight, withers height and body condition score for all heifers at 3 key events in the study; the onset of puberty, first service and conception. There were no statistical differences between dietary groups for any of the events.

Event / Measure	Puberty onset	First Service	Conception
Body weight (kg)	289.9 (281.6 - 298.2)	414.2 (406.0 - 422.3)	440.5 (428.6 - 452.4)
Withers height (cm)	123.0 (121.8 - 124.2)	133.5 (132.7 - 134.3)	135.2 (134.3 - 136.1)
BCS	2.91 (2.85 - 2.96)	3.07 (3.02 - 3.12)	3.10 (3.04 - 3.23)

Survival Analyses

Puberty, first service and conception: Kaplan-Meier survival plots and Cox regression demonstrated a trend for heifers from Group A to reach puberty (lr test P = 0.481, Hazard ratio 1.14, 95% CI 0.77 - 1.72), first service (lr test P = 0.062, Hazard ratio 1.47, 95% CI 0.97 - 2.22) and conception (lr test P = 0.182, Hazard ratio 1.32, 95% CI 0.88 - 1.98) at an earlier age than calves in Group R (Figure 6.3).

Visual appraisal of survival curves for age at puberty onset, age at first service and age at conception suggested the greatest differences between dietary groups in time to achieving the 3 targets, occurred after the median (50%) 'survival' point had been met. Data for animals that were in the most 25% and least 25% rapid (upper and lower quartiles) of the cohort reaching these targets were segregated for further study.

Comparison of the upper and lower quartiles of age at puberty data showed no significant association with any of the measured variables (Table 6.7). However calves in the upper quartile for both age at first service and age at conception had higher birth weights ($P < 0.05$) whilst higher plasma TP and *ad libitum* milk feeding were both associated ($P > 0.05$) with an earlier age at first service. There was a trend for calves with higher BCS at 3 weeks of age to achieve first service earlier (P = 0.058, Table 6.7).

Output from the multivariable Cox regression model fitted to explore the relative contribution of potential explanatory variables identified during the analysis of the upper and lower quartiles is presented (Table 6.8). It was apparent that whilst dietary group had the largest impact, plasma TP was also important whilst birth weight had a small but significant impact.

Pre- determined Key Performance Indicators: There were no statistically significant differences in the survival function between dietary groups for either the body weight (P = 0.088, Hazard ratio 1.42, 95% CI 0.94 - 2.16) or withers height survival curve. (P = 0.309, Hazard ratio 1.22, 95% CI 0.82 - 1.82; Figure 6.4).

Table 6.7: Mean birth weight, plasma TP at 48 hours and BCS at 3 weeks of age for calves in the upper and lower quartiles of survival curves for puberty, first service and conception. The effects of disease and dietary group (*ad libitum* or restricted MR) during the first 12 weeks were also assessed. The number of animals with disease and number of animals in pre-weaning Group A in the top and bottom quartiles are presented.

Event	**Puberty**			**First service**			**Conception**		
Outcome variable	**Upper quartile**	**Lower quartile**	**P Value**	**Upper quartile**	**Lower quartile**	**P Value**	**Upper quartile**	**Lower quartile**	**P Value**
Birth weight (kg)	41.7	42.5	0.299	45.8	40.8	0.002	45.3	39.1	<0.001
Plasma TP (g/L)	6.89	6.94	0.405	7.10	6.68	0.036	7.04	6.83	0.192
BCS (at 3 weeks)	2.60	2.64	0.328	2.72	2.56	0.058	2.73	2.63	0.178
Disease (1st 12 weeks)	21 (67.7%)	15 (55.5%)	0.340	9 (34.6%)	6 (24.0%)	0.406	9 (36%)	8 (32%)	0.765
***Ad libitum* pre-weaning group (*n*, %)**	18 (58.1%)	10 (37.0%)	0.110	15 (57.7%)	7 (28.0%)	0.032	13 (52%)	10 (40%)	0.395

Table 6.8: Cox regression model to assess the impact of selected explanatory variables on the age at timing of first service in the study cohort. The hazard ratio may be taken to indicate the probability of first service occurring in Group A heifers compared to Group R at a point in time.

Explanatory variable	**Hazard Ratio**	**95% CI**	**P value**
Pre-weaning dietary group	1.51	0.99 - 2.30	0.054
birth weight	1.05	1.01 - 1.10	0.009
plasma TP	1.36	1.05 - 1.76	0.019

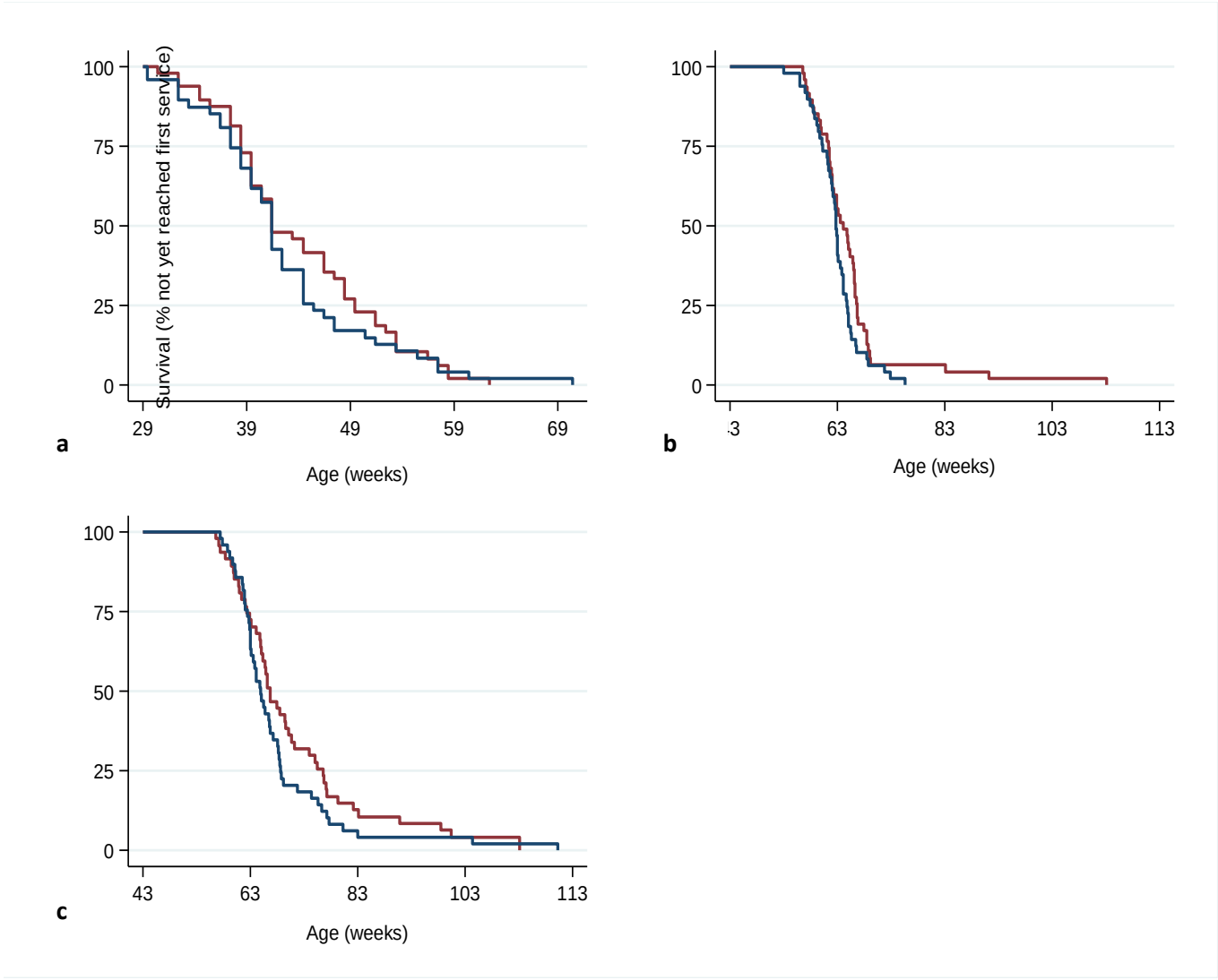

Figure 6.3: Kaplan- Meier survival curve for proportion of heifers not yet reaching a) puberty, b) first service and c) conception in Group A (blue line) and Group R (red line).

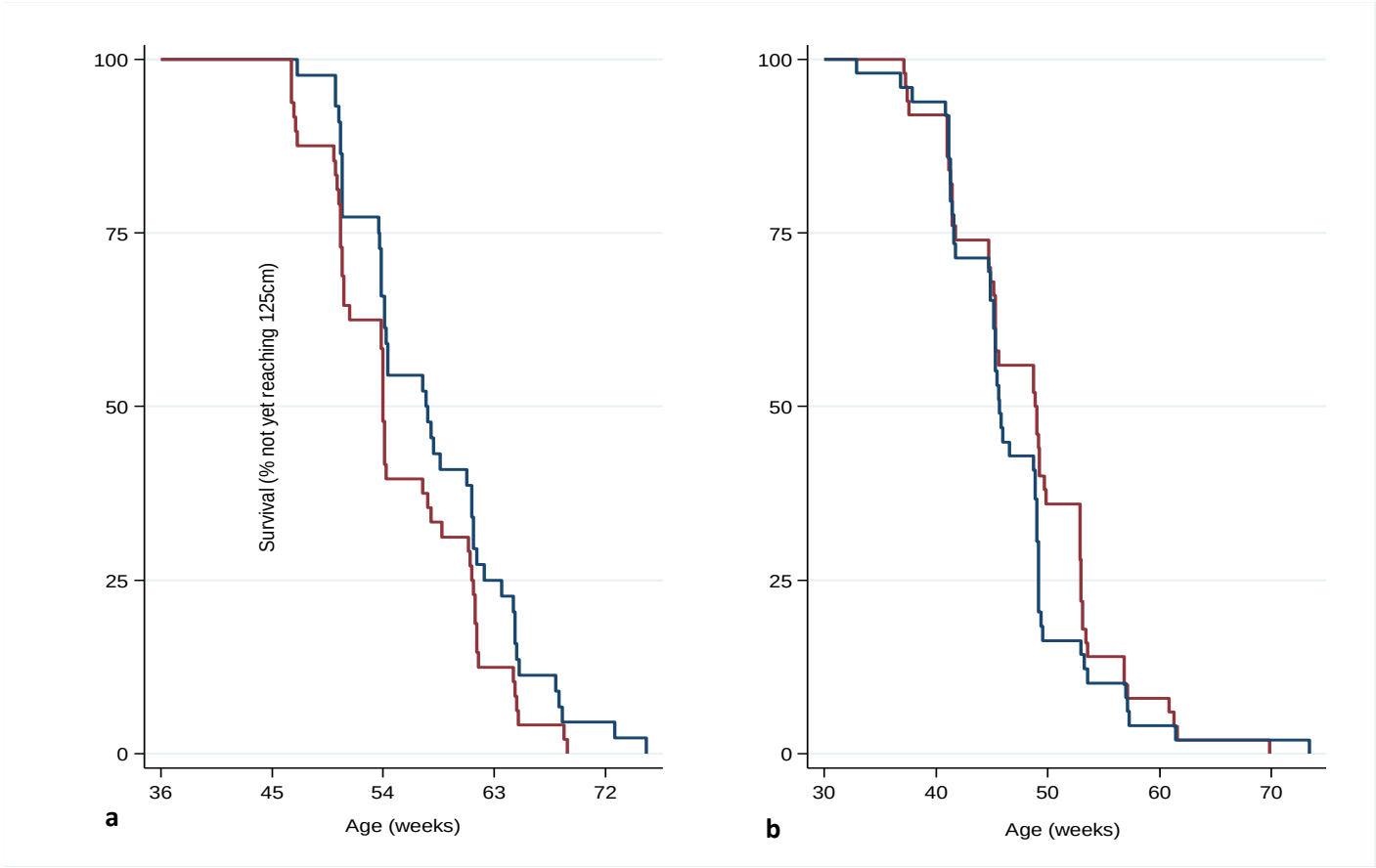

Figure 6.4: Kaplan- Meier survival curve for proportion of heifers not yet reaching a) 380kg body weight and b) 125cm withers height in Group A (blue line) and Group R (red line).

IGF-1 Concentrations

Univariable analyses: Plasma IGF-1 concentrations increased for all calves from birth to 58 weeks. The mean plasma IGF-1 concentration at 48 hours of age (42.36µg/L, 95% CI 35.19 - 49.52) did not differ between dietary groups. However, by 3 weeks of age a marked difference in plasma IGF-1 was apparent between dietary groups, with mean values for Group A heifers being approximately 250% greater than Group R animals, (Table 6.9). Group differences in plasma IGF-1 concentrations persisted at 20 weeks of age but statistical significance was lost by the time heifers reached 58 weeks of age.

In descending order of magnitude, body condition score, dietary group, withers height, loin height, crown rump length and body weight, were all positively associated with plasma IGF-1 concentration, whilst disease during the pre-weaning phase was negatively associated (Table 6.10).

Multivariable analyses: Explanatory variables that remained in the final plasma IGF-1 concentration model were: pre-weaning dietary group (*ad libitum* or restricted MR) with an interaction with age in weeks, plasma TP concentration, BCS, with calf identity as a random effect (Table 6.11, Figure 6.5).

Whilst there was a moderate association between plasma IGF-1 concentration at 3 weeks of age and ADG from birth to 3 weeks (Figure 6.6a, $R^2 = 0.44$, $P<0.001$), the association between IGF-1 at 3 weeks of age and overall ADG (birth to 58 weeks) was very weak (Figure 6.6b, $R^2 = 0.08$, $P<0.048$). There were no associations between IGF-1 concentration and ADG at any other time points studied.

Table 6.9: Mean plasma IGF-1 concentration and 95%CI for calves in pre-weaning Group A (*n* = 25) and Group R (*n* = 25) at 48 hours, 3 weeks, 20 weeks and 58 weeks of age.

Age (weeks)	Mean IGF-1 Concentration (µg/L)		P Value
	Group A (95% CI)	Group R (95% CI)	
0	46.94 (35.18 - 58.70)	37.78 (29.10 - 46.46)	0.101
3	73.93 (59.54 - 88.33)	28.87 (20.53 - 37.21)	<0.001
20	114.11 (98.28 - 129.93)	91.71 (78.34 - 105.08)	0.015
58	141.91 (126.24 - 157.58)	129.57 (117.28 - 141.85)	0.103

Table 6.10: Regression analyses to evaluate explanatory variables associated with changes in plasma IGF-1 concentrations. The regression equations include age (in weeks) in addition to the variable in question but are unadjusted for other variables. Results of individual analyses are presented together in one table (full models are presented in Appendix E, Table E.9).

Outcome variable: IGF-1 concentration			
	Coefficient	95% CI	P Value
Birth weight	-0.754	-1.686 - 0.177	0.112
Body weight	0.339	0.053 - 0.625	0.020
Plasma TP	3.539	-3.475 - 10.552	0.321
Withers height	2.011	1.211 - 2.810	<0.001
Loin height	1.656	0.805 - 2.507	<0.001
HFL	0.965	-0.332 - 2.263	0.144
CRL	0.992	0.359 - 1.625	0.002
BCS	40.023	24.158 - 55.888	<0.001
Diarrhoea or pneumonia (1st 12 weeks)	-30.250	-56.953 - -3.547	0.027
Pre-weaning dietary group (Group A or Group R)	23.060	13.064 - 32.436	<0.001

Table 6.11: Multivariable regression model of the association between IGF-1 concentration and dietary group, including an interaction term between age (in weeks) and pre-weaning dietary group. Other confounding variables were included and calf identity was entered as a random effect. The residual variance attributed to individual calf identity was 28.31% (95% CI 14.11 - 48.69).

Outcome variable: IGF-1 concentration	**Coefficient**	**95% CI**	**P value**
week 3	-4.467	-21.589 - 12.656	0.609
week 20	46.337	30.625 - 62.048	<0.001
week 58	80.077	63.827 - 96.326	<0.001
dietary group (*ad libitum* or restricted MR)	5.088	-14.943 - 25.119	0.619
week3#*ad libitum* MR	33.716	9.151 - 58.280	0.007
week 20#*ad libitum* MR	17.878	-4.963 - 40.720	0.125
week 58#*ad libitum* MR	10.143	-12.645 - 32.931	0.383
BCS	14.484	-0.471 - 29.440	0.058
plasma TP	8.926	0.632 - 17.221	0.035
constant	-60.871	-132.517 - 10.774	0.096

Random-effects Parameters	**Estimate**	**95% CI**
calf	239.945	111.674 - 515.568
Residual	607.639	469.036 - 787.199

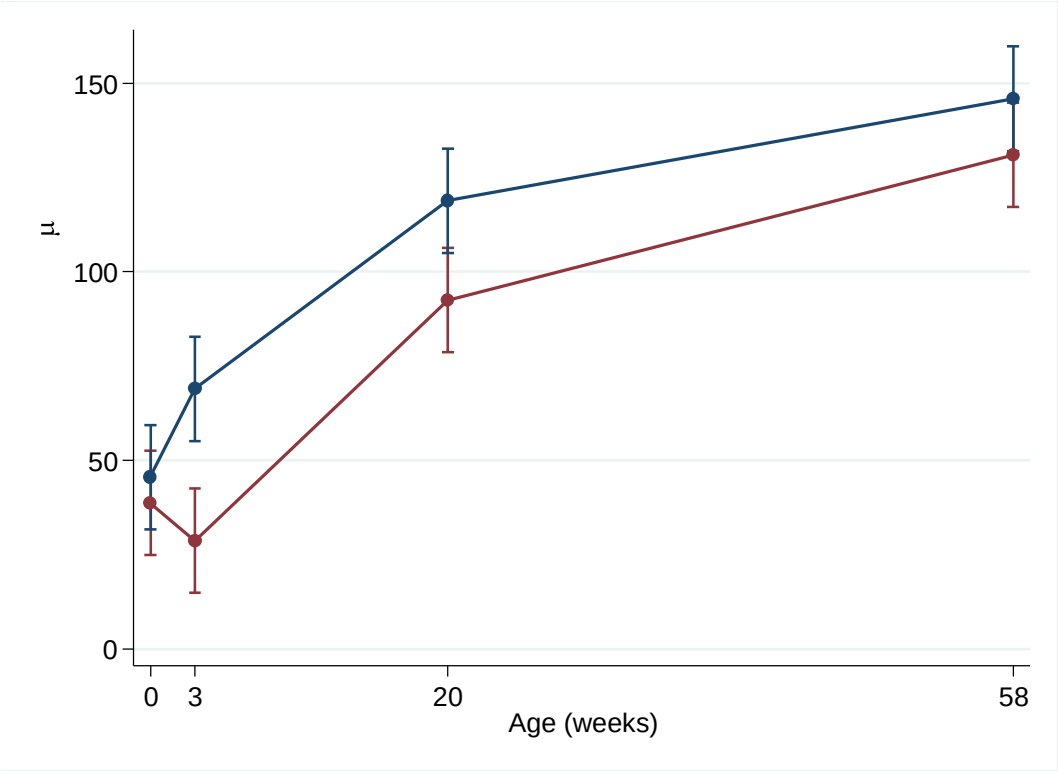

Figure 6.5: Unadjusted marginal means (95% CI) of predicted IGF-1 concentration (µg/L) for calves in Group A (blue line) and R (red line) at 48 hours (0 weeks), 3 weeks, 20 weeks and 58 weeks of age.

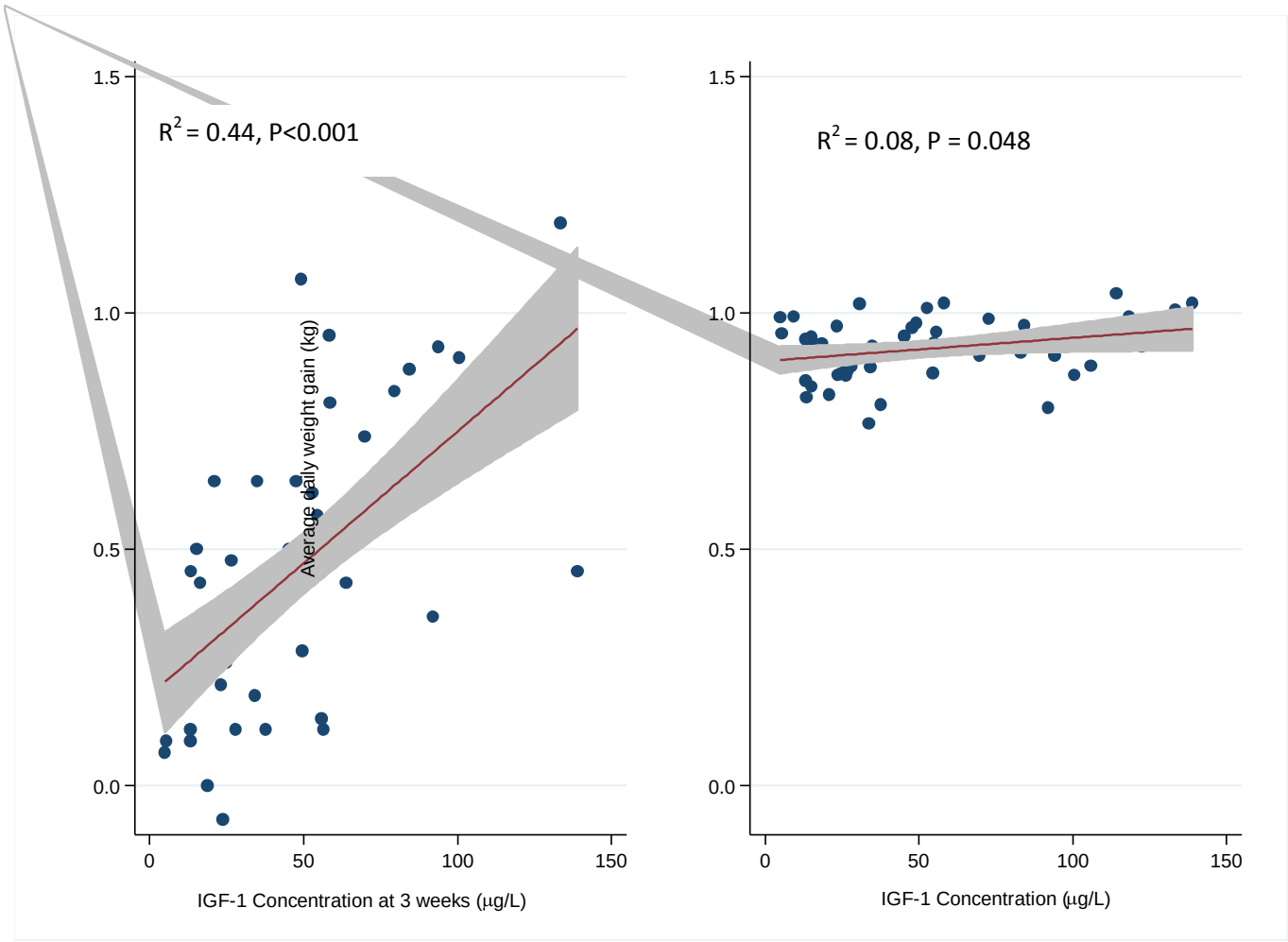

Figure 6.6: Linear regressions of IGF-1 concentration at 3 weeks of age and average daily body weight gain for calves from a) birth to 3 weeks and b) the whole period (birth to 58 weeks). The coefficients of determination (R^2) are displayed above the regression line (red line) with grey shaded areas indicating 95% CI.

6.4 Discussion

There were no dietary group differences in the average daily weight gains of heifers from the onset to the end of the study. However, the advantage in body weight that Group A animals had gained over Group R during the first 3-4 weeks of life (Thesis Chapter 3) was maintained throughout both the pre-weaning and post-weaning periods; Group A heifers were 10-15 kg heavier than Group R animals at any given time point between 12 and 80 weeks. These data highlight the importance of appropriate neonatal nutrition to allow for optimal long term growth and is supported by other research groups internationally (Bach, 2011; Davis Rincker et al., 2011; Hepola, 2003; Moallem et al., 2010; Morrison et al., 2012; Van Amburgh, 1998).

Although the ability for 'catch-up' or 'compensatory' growth following a period of limited nutrient availability is well documented for various mammalian species (Tanner, 1981; Wilson and Osbourn, 1960), no evidence of this was observed for Group R heifers throughout this study. Abdalla *et al* (Abdalla et al., 1988) demonstrated that compensatory growth occurred in Holstein calves after a period of restriction of protein and energy intake that supported 50% of the daily body weight gain of non-restricted study animals for between 112 and 154 days. Calves in the cited study were at least 8 weeks of age at the onset of restriction. By contrast, in the present study half the animals had restriction to MR from birth. It has been argued that compensatory growth cannot occur if dietary restriction is imposed during very early life (Lawrence et al., 2012; Van Amburgh et al., 2011); the current study supports this hypothesis and highlights the long term impact of early life nutrient restriction in the dairy calf which may have important considerations for life time production targets.

The impact of pre-weaned dietary group on all measures of growth extended into the post-weaned period, irrespective of identical nutrition and management during the post-weaned period (12 weeks of age onwards). Dam parity also had an important association with most morphometric measures. It is recognised that heifers, having not reached full body size at first calving, generally give birth to smaller calves than multiparous cows (Holland and Odde, 1992). Calves that are larger at birth may have an advantage over smaller calves with the ability to consume larger volumes of colostrum and MR than their smaller counterparts. In

the present study this would only be the case if they had belonged to Group A since MR intakes were restricted for calves in Group R and animals were housed individually for the first 3 weeks of life. It may be argued that larger calves could eat more concentrate feed than smaller calves, however concentrate intakes were not different between dietary groups during the first 3 weeks of life (Chapter 3). Although it was beyond the scope of the current study, the association between dam parity (which may be linked to birth weight) with morphometric measures of growth may be due to sire selection or genetic imprinting/epigenetic effects that occurred whilst *in utero* (Funston and Summers, 2013). Maternal nutrition has also been reported to have an impact on the performance, health and reproductive efficiency of progeny (Funston and Summers, 2013; Holland and Odde, 1992). We were unable to investigate this.

Whilst differences in body weight between the 2 dietary groups were apparent for the duration of the study, the trend was not consistent for other measures, and dietary group differences in morphometric measures tended to disappear over time. In a study conducted by Shamay *et al* (Shamay et al., 2005), female Holstein calves (*n* = 40) with 30 minute twice daily *ad libitum* access to whole milk compared to restricted MR had increased body weights (16kg) and greater skeletal growth during the pre-weaning period. During the post-weaned period all calves were fed similarly until 180 days of age (13.5% CP). From 180 to 270 days the 2 pre-weaning dietary groups were further divided to form 4 dietary groups. Half of the whole milk-fed and half of the MR fed calves were fed supplementary protein (2% fish meal in addition to the 13.2% CP of a growing heifer ration). Whole milk fed calves remained significantly heavier until first calving but the differences in skeletal growth had disappeared. Calves in Shamay *et al's* (Shamay et al., 2005) *ad libitum* whole milk fed group reached puberty 23 days earlier than the restricted MR fed calves. These data are remarkably similar to those of the current study. However, the whole milk, protein supplemented calves produced 771kg more milk during their first lactation than MR fed calves. This would suggest that the benefits of *ad libitum* whole milk feeding during the pre-weaning period could be diminished by sub-optimal post weaned, pre-pubertal nutrition.

In the present study, the BCS of animals in both dietary groups increased after about 36 weeks of age. The optimal BCS for an adult Holstein cow is approximately 3 - 3.5 (mid-late lactation) on a 1-5 scoring system (Edmonson et al., 1989; Kellogg) and it is widely

recognised that both low and high BCS recordings are associated with reduced fertility and ultimately lower productivity (Bisinotto et al., 2011; Kellogg; Sinclair, 2010). Heifers from the current study had a mean BCS of 2.9 at the onset of puberty and 3.1 at pregnancy. However, observation of individual animals not achieving pregnancy at first or second service would suggest a trend for BCS to increase as they aged but still remained on the study under bulling heifer management conditions (nutrition). An example of this would be one animal that achieved pregnancy at 105 weeks after 12 services with a BCS of 4.8. It has been reported that animals who conceive later with a high BCS have an increased risk of dystocia at calving (Hoffman et al., 1996); thus excessive BCS during pregnancy should be avoided. The increased BCS with time recorded in this study may be due to the highly variable post-weaning diet, where leftovers from milking and dry cow diets were likely to contain inappropriately increased crude protein and energy content for growing heifers. If animals failed to conceive to their first or second service, they remained in the bulling heifer housing for longer and were therefore exposed to this inappropriate diet for a prolonged period.

Most U.K. dairy farmers do not routinely weigh their growing heifers (Thesis Chapter 2) and often base decisions for timing of first service on the approximate height or overall general stature of the individual animal. This study emphasises the deleterious negative feedback loop between failure to conceive and increased BCS and the importance of using a more robust system to optimise the timing of first service. This requires a combination of factors including body weight, height and BCS monitoring and appropriate heat detection/management.

It has been reported that the age at which heifers reach puberty is associated with body weight gain from birth to puberty (Day et al., 1986; Patterson et al., 1992). On this basis it could be anticipated that heifers from Group A would attain puberty earlier than those from Group R. In agreement with this, heifers in Group A attained puberty over 2 weeks earlier ($P = 0.096$), conceived 2.3 weeks earlier ($P = 0.171$) and were submitted for first insemination significantly earlier (2.6 weeks, $P = 0.038$). It may be speculated that the failure to achieve statistical significance for age at puberty and conception is a function of the relatively small sample size in the present study. Furthermore, timing of first service and conception are dependent not only on the animal's performance but also on the farm management with regards to heat detection and if that animal is eligible for service. In fact, the farm

management failed to achieve the set KPI targets as evidenced by observations that mean withers height and body weight for heifers at first service were 133.5cm and 414kg. This suggested that the opportunity to benefit from the improved growth in Group A heifers was not fully exploited. Despite this failure of management, the mean predicted age at first calving for heifers in this study was 25.3 and 25.9 months for Group A and R respectively, demonstrating a benefit equivalent to approximately 2 weeks of milk production for animals in Group A. Visual inspection of the survival curves for attainment of KPI's (withers height ≥ 125 cm, body weight ≥ 380 Kg) confirmed that in the case of both height and weight, the difference between median 'survival' was in the region of 4-5 weeks. This would suggest that if management had fully utilised the growth advantages, the median difference in attaining first service could have been within this time frame (significantly greater than actually observed). From these findings, we can conclude that if growth benefits are to be exploited maximally, then careful management to ensure that animals are served as soon as they reach target weight and height must be engaged.

Long term studies are beginning to provide evidence that young calf feeding and management has a lifelong impact on the health and performance of these animals as dairy cows (Bach, 2011; Bar-Peled et al., 1997; Shamay et al., 2005; Van Amburgh et al., 2011). This begins as early as ensuring correct colostrum feeding to achieve success of passive transfer of immunoglobulins (Faber et al., 2005; Lago et al., 2006; Macdonald et al., 2007) along with benefits from other non-immunoglobulin colostrum components (e.g. highly digestible nutrients, hormones and growth factors) that are thought to be important for growth and long term performance and act independently of passive transfer of immunoglobulins (Faber et al., 2005; Van Amburgh et al., 2011).

In the current study, plasma TP concentration measured at 48 hours of age was shown to have a significant positive impact on growth from birth to conception. Interestingly, we were unable to demonstrate any association between plasma TP and early life growth up till 12 weeks of age (Chapter 3). Such was the impact of plasma TP on long term growth that it was a significant explanatory variable in the Cox regression model for time to first service. This was clarified by further study of animals from the upper and lower quartiles with animals in the upper quartile having significantly ($P < 0.05$) higher plasma TP concentrations (mean: 7.10 g/dL) compared to those in the bottom quartile (mean: 6.68 g/dL). This impact

could have economic benefits in terms of animal lifetime production and these observations would support other recent studies assessing the long term impact of colostrum ingestion (Faber et al., 2005; Van Amburgh et al., 2011).

As has been described in Chapter 3, there was a relatively high incidence of both diarrhoea and pneumonia in the study cohort, with higher disease incidence in Group A compared to Group R. Occurrence of both diarrhoea and pneumonia were negatively associated with growth during the post weaning phase, although neither event was identified as a significant variable in any of the analyses regarding time to puberty, first service or conception. It has been well documented that respiratory disease during the pre-weaned period has an immediate negative effect on animal welfare, increases microbial usage and drives up production costs due to treatment (Gorden and Plummer, 2010). The long term impact of early life disease on performance may include lower growth rates in later life (Bach, 2011; Lago et al., 2006), decreased productivity (Bach, 2011), increased likelihood of death prior to first calving (Waltner-Toews et al., 1986) or increased age at first calving (Correa et al., 1988). All disease is costly and should be minimised where possible, however it has been reported that neonatal diarrhoea has little effect on the long term productivity of Holstein heifers, with no impact on the probability of completing the first lactation (Bach, 2011).

It has been documented that circulating plasma IGF-1 concentrations are closely related to body weight during the pre-pubertal period in Holstein calves (Macdonald et al., 2007). During this study the IGF-1 concentration of heifers in pre-weaning Group A was higher than Group R animals at 3 and 20 weeks of age and a positive association between IGF-1 concentration and body weight was identified during univariable analysis. Curiously, multivariable analysis suggested that BCS was strongly correlated with IGF-1 concentration, along with plasma TP at 48 hours of age (to a lesser magnitude). It was not predicted that BCS would have such a large positive impact on IGF-1 concentration. However, BCS may be used as an indirect measure of extreme nutritional status in healthy young dairy calves and it is widely accepted that IGF-1 concentration is influenced strongly by nutrition (Bartlett et al., 2006; Hammon et al., 2000). It was confirmed in Chapter 3 that Group R calves, as well as having lower body weight at 3 weeks of age, also had lower BCS than Group A animals. As we often describe restricted MR fed calves as being held in a state of ‘chronic hunger’ (Thesis Chapter 3), this may in part explain the importance of BCS in the IGF-1 model. The

positive relationship between plasma TP and IGF-1 concentration may be similar to the previously discussed point on plasma TP (at 48 hours) and subsequent growth.

The association between pre-weaning dietary group and plasma IGF-1 concentration has been observed in a previous study by Brickell *et al* (Brickell et al., 2009b), where calves offered *ad libitum* MR during the pre-weaning period had higher plasma IGF-1 concentrations at 1 and 6 months compared to restricted MR fed calves; the results of the current study support this, with similar findings at similar sample ages.

In addition to body weight, circulating IGF-1 concentration has been associated with growth rate in calves (Kinsbergen et al., 1994). In this study, higher IGF-1 concentrations for Group A calves at 3 weeks of age coincided with increased growth rates compared to Group R heifers during this period (Figure 6.13a). Whilst Group R animals had a clear non-compensation of body weight throughout the study period (Figure 6.1a), compensation did occur in terms of other morphometric measures. During multivariable analyses of IGF-1 concentration however, inclusion of morphometric variables was not appropriate as they resulted in poor model fit. The suggestion that IGF-1 concentration may be used as a predictor for subsequent growth has been made by others (Brickell et al., 2009b). Unfortunately, results from the present study are not able to support this hypothesis since the association between IGF-1 concentration at 3 weeks and subsequent ADG, although statistically significant was very small in magnitude (Figure 6.13b, R^2=0.08, P=0.048).

Faster growing Holstein heifers achieve puberty earlier (Sejrsen, 1994), concentrations of circulating IGF-1 becomes maximal at puberty and starts to decrease between 15 to 18 months of age (Kerr et al., 1991). As plasma IGF-1 concentrations were greater for Group A heifers at 20 weeks of age, it would be fair to predict that these animals were advancing into reproductive maturity at a faster rate than Group R heifers. This was confirmed by determination of puberty onset at 2.5 weeks earlier for Group A heifers than for Group R (P = 0.096).

The results obtained from this study suggest that circulating IGF-1 concentration of heifers at specific points throughout the rearing period gives a key insight into the nutritional status of the animal at that time but unfortunately, is not a useful predictor of past or future growth of Holstein heifers.

This study confirms that correct neonatal nutrition of Holstein heifer calves plays a major role in ensuring profitability within a dairy enterprise. Allowing these animals access to *ad libitum* MR from birth to 12 weeks enabled Group A calves to gain approximately 15kg more body weight than Group R animals, and that this weight advantage was gained during the first 3 to 4 weeks of life. Compensatory growth (as measured by body weight) of Group R animals did not occur, resulting in Group A animals achieving an earlier age at puberty onset, first service, conception and therefore entry into the milking herd. Although these differences were not always statistically significant, management to ensure achievement of KPI's for first service will allow for major financial benefits to the dairy farmer through reduced age at first calving and thus entry into the milking herd.

6.5 References

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Chapter 7

Final Discussion

7.1 Final Discussion

Over the last 30 years U.K. dairy herd size and lactational yield have increased, while herd numbers have decreased. The financial pressures exerted upon U.K. dairy farms, in terms of farm-gate milk prices, have forced producers to either move away from dairying altogether or to reduce their cost of production. In parallel to this, there has been resurgence of interest in dairy calf management, specifically health and welfare issues. The challenge of decreasing the cost of production while increasing animal welfare has been difficult, but there is increasing research output that has attempted to address the issue (Appleby et al., 2001; Bach et al., 2010; Borderas et al., 2009). The importance of minimising mortality rates in pre-weaned calves has long been recognised (Ortiz-Pelaez et al., 2008), and there has also been a realisation that the age at first calving can have a considerable impact on lifetime profitability (Brickell et al., 2009). Based on previous research, the target for age at first calving (AFC) for dairy heifers is currently 23 – 24 months (Hare et al., 2006). Currently the mean age at first calving in the U.K. is reported to be greater than 26 months (Brickell et al., 2009), representing a significant financial loss. The impact of early life nutrition on future milk production and its possible epigenetic roots has recently been recognised, providing further impetus for research (Soberon and Van Amburgh, 2013; Van Amburgh et al., 2011).

The current study was designed to investigate and compare the impact of *ad libitum* milk replacer (MR) feeding under commercial conditions with calves group housed from birth ($n \leq 6$/group). For comparison, a control group with calves housed from birth until 3 weeks and thereafter grouped ($n \leq 6$) and fed restricted MR according to current U.K. practices was also studied.

There is a dearth of information regarding current dairy calf rearing practices in the U.K., although recent work has helped establish baseline performance data (Brickell and Wathes, 2011). A current trend in the milk retail sector is the establishment of producer networks whereby farmers are contracted to solely supply liquid milk to one retailer. Tesco Plc has established one such group - the Tesco Sustainable Dairy Group (TSDG). The retailer for whom the farmers produce the milk, impose extra animal welfare and management demands upon their supply chain members. It is anticipated that setting these contractual obligations will improve animal health and welfare and ensure a high quality product. It is

hypothesised that these high standards will further drive improvement in animal welfare across the dairy industry.

The first objective of this thesis was to describe current dairy calf rearing practices and investigate the hypothesis that TSDG membership resulted in measurable differences in either farm performance or management practices compared to non TSDG farms. A postal questionnaire to assess calf rearing practices was developed and delivered to a total of 1000 TSDG and non-TSDG farmers. The response rate was high (72%) and gave confidence that the results were representative of the U.K. dairy industry. Overall, the results suggested that there is considerable variation in management practices on dairy farms across the U.K. There was a wide variation in calving management and colostrum delivery; only just over half of the farms ensured calves received at least 3 litres of colostrum within 6 hours of birth. The lack of a suitable colostrum management policy on many farms undoubtedly represents a major deficit in current U.K. dairying practices. Only 6% of farmers reported regular monitoring of calf immune status, suggesting a lack of recognition of the importance of adequate colostrum feeding. Almost 85% of farmers fed restricted volumes of milk or MR on a twice daily basis and only a minority of farmers fed more than 6 litres of MR daily. This was a disappointing finding in light of the increasing evidence base regarding the benefits of increased milk or MR feeding (Soberon et al., 2012). Similarly, less than 1% of farmers reported regular monitoring of growth.

Within the questionnaire study (Chapter 2), there were few overall significant differences between TSDG and non-TSDG farms in terms of calf and heifer management. This suggested that membership of a producer network does not result in improved management practices. This questionnaire provided a valuable snapshot of dairying practices in the U.K. in 2012. A limitation of this study was that no morbidity or mortality data was collected and estimation of the impact of rearing practices on calf health and performance was not possible. This data was not collected since it is generally recognised that few farmers record such data accurately or would be reluctant to provide this information, resulting in potentially biased results.

The third chapter addressed the primary study objective, which was to evaluate growth and health of Holstein heifer calves within a commercial setting. This was achieved *via* an

intervention study carried out over a two year period on a single commercial dairy farm of 170 high yielding Holstein dairy cows. One hundred female calves were recruited over a 21 month period and allocated to one of two feeding strategies at birth. Calves were reared in groups of up to 6 animals per group, with an age range of no more than 2 weeks. It was not possible to randomise calves to treatment groups; this represents a study weakness but one that could not be avoided. The chosen intervention was *ad libitum* MR feeding with restricted MR feeding as a control. Calves in the control group were fed and managed according to the standard farm policy, which was to feed calves 2.5 litres of MR twice daily from birth to 3 weeks, then 3 litres twice daily until weaning. These volumes are considerably greater than many of the reported volumes provided during the questionnaire study (Chapter 2). This suggested that feeding practice on the study farm was above average for the U.K. dairy industry. The decision to base the intervention on *ad libitum* MR feeding rather than increased volumes was made on the basis that it was important to understand the growth potential of Holstein calves with access to unlimited MR. It was not the intention of the study to provide a novel "off the shelf" feeding strategy for adoption by the farming industry.

Calves born from heifers were smaller than those born from multiparous dams. In addition, regression modelling suggested that there was a seasonal impact on calf birth weight, with calves born during the summer months weighing up to 2kg greater than if born during winter. In contrast, birth weights of calves born in tropical climates have been higher in spring than in autumn (Odde et al., 1985). Heat stress in summer months during gestation has been found to cause a reduction in birth weight of calves in the summer and autumn (Bonsma, 1949); a similar situation is observed in dairy cattle in the southern states of the U.S.A. (Van Amburgh, 2014). All the cattle in the present study were housed year round, apart from a short grazing period in the summer. They were all offered the same diet throughout the year, thus dam nutrition is unlikely to account for the seasonal differences in calf birth weights. It may be hypothesised that birth weight is a reflection of an inherent seasonality in the bovine; however larger studies would be required to confirm this.

During this study all calves were monitored for adequacy of colostrum intake *via* measurement of plasma total protein concentrations (PTP) at 48 hours of age. Additionally, all colostrum was assessed for quality using a Brix refractometer. All colostrum was

measured as being good quality (>22%), suggesting that on the study farm colostrum quality was not an issue (Bielmann et al., 2010). Results of these findings were not available to farm staff in order to avoid any bias in colostrum administration. There were no cases of failure of passive transfer (FPT), with 48 hour PTP concentrations of greater than 5.5g/dL for all calves. The 100% success rate of passive transfer of immunoglobulins may have been due to an indirect researcher effect. Anecdotal evidence suggests that since the end of this intervention study, FPT has been identified in calves on the study farm. Throughout this study there were no dam parity effects on colostrum quality; this supports evidence from other work that suggests colostrum from heifers is of equal quality to colostrum from multiparous animals (Godden, 2008; Gulliksen et al., 2009).

A key study finding was the dramatic dietary group difference in growth rate during the first 3 weeks of life. *Ad libitum* MR fed calves grew at a rate of 0.72kg (95% CI 0.61-0.82) per day compared to 0.17kg (95% CI 0.08-0.26) per day in the restricted MR fed calves during this period. This minimal growth for restricted MR fed calves was accompanied by a dramatic loss in body condition score (BCS) of nearly 0.5 BCS points over the first 4 weeks, which is comparable to that of an adult lactating cow during the first 8 to 10 weeks *post-partum*. In contrast, a consistent increase in BCS from birth was recorded in the *ad libitum* MR fed animals. While growth rates increased for restricted MR fed calves after 3 weeks of age to a rate that was similar to that of the *ad libitum* MR fed calves, their body weights remained lower and no compensatory growth was observed throughout the pre-weaning period. This is an important finding as other authors have reported compensatory growth of Holstein calves after a previous period of nutritional restriction (Abdalla et al., 1988). In the current study, this severe restriction of growth during the first three weeks of life has both welfare and economic implications. The Animal Welfare Act (2006) states that the five needs of animals should be met by providing a suitable environment, a suitable diet, allowing animals to exhibit normal behaviours, housing animals with or apart from other animals and protecting animals from pain, injury, suffering and disease (DEFRA, 2006; Odde et al., 1985). In this study, restricted MR fed calves were not being provided with a suitable diet appropriate to their needs during the first 3 weeks of life, as demonstrated by the loss in BCS and minimal growth compared to their *ad libitum* fed counterparts. In addition to the differences in body weight gain, other morphometric measures of growth differed between

dietary groups. Changes in withers and loin height, heart girth, crown to rump length and hock-fetlock length corresponded broadly to changes in body weight measures and were significantly higher for *ad libitum* MR fed calves. Recording morphometric measures of growing calves can be a useful tool for farmers when making management decisions. A number of morphometric measures were recorded in the current study. This was a time consuming task and many measures gave the same information. The most useful measures recorded were body weight, withers height, heart girth and BCS in the study animals. Many farms do not have access to weigh scales; an alternative to this would be to record heart girth measures to monitor growth. The correlation between body weight and heart girth of animals in this study was very high (R^2 0.97) suggesting it is a valid proxy for body weight.

Restricted feeding of milk or MR has traditionally been justified on the basis of promoting early rumen development associated with consumption of concentrate feed at an early age (Quigley et al., 1991). New born calves are mono-gastric with undeveloped rumens and adaption to a new diet is generally accepted to take about three weeks. Thus it is highly unlikely that the rumen of a calf under 3 weeks of age can play any role in digestion of consumed concentrates. In fact, concentrate intakes during this period were minimal in calves of both dietary groups (Chapter 3). It therefore seems illogical to expect dairy calves of less than 3 weeks of age to partially acquire their nutrient requirements from concentrate feed, implying that under-feeding of MR at this stage is not compatible with the concept of a "suitable diet" as per the five needs (DEFRA, 2006; Odde et al., 1985). The BCS loss observed in restricted MR fed calves during the first few weeks of life is of interest since results from the carcass dissection study (Chapter 4) suggested there was no sub-cutaneous fat present in calves of either dietary group at this age. The loss of BCS in the restricted MR group likely represents the mobilisation of lean tissue (and intra-muscular adipose tissue) in order for the calf to access energy for maintenance. While overall, the pre-weaning growth of restricted MR fed calves was in line with current dairy calf growth targets (0.72kg daily), the severe under-nutrition experienced in the first three weeks of life may have an impact in later life *via* currently unknown epigenetic mechanisms (Van Amburgh et al., 2011).

A concern raised with regards to feeding increased volumes of milk or MR is that calves will have poor rumen development at the time of weaning due to insufficient consumption of concentrate feed (Jensen and Budde, 2006; Quigley et al., 2006; Webb et al., 1969). In the

present study, a three week step-down weaning strategy was adopted for the *ad libitum* MR fed calves in order to avoid this problem. Concentrate intakes in *ad libitum* MR fed animals was relatively low prior to the onset of weaning, but increased rapidly to that of restricted MR fed calves by the end of the pre-weaned period with no adverse effects. Although the carcass dissection study (Chapter 4) showed greater rumen-reticulum weights for restricted MR fed calves at 3, 9 and 12 weeks of age compared to *ad libitum* MR fed animals, there were no dietary group differences in gross appearance of rumen papillae at 9 weeks of age.

In the present study, disease incidence during the pre-weaning period was high; 80% of animals underwent at least one disease episode even though, based on calf 48 hour PTP concentrations, there was no evidence of FPT. This finding highlights the limitations of passive transfer of immunoglobulin as a sole determinant of disease risk (Gutzwiller, 2002) and re-enforces the concept that disease is multi-factorial involving infectious agent, host and environment (Lorenz et al., 2011; McGuirk, 2007; Roy and Ternouth, 1972; Smith, 2003). The risk of both diarrhoea and pneumonia in study calves was significantly greater in those fed *ad libitum* MR. In the case of diarrhoea, two hypotheses may be generated to explain this observation. The first is that consumption of increased volumes of MR is a risk factor for diarrhoea. While increased milk or MR feeding is frequently believed by farmers and veterinarians to be associated with increased disease risk, peer reviewed studies do not support this hypothesis (Appleby et al., 2001; Bartlett et al., 2006; Diaz et al., 2001; Jasper and Weary, 2002; Nonnecke et al., 2003). The second hypothesis is that group housing of the *ad libitum* MR fed calves facilitated transmission of infectious agents *via* direct contact between animals and by increased environmental contamination. This is supported by studies which show a significant increase in disease risk associated with group housing (Gorden and Plummer, 2010; Tomkins, 1991).

The overall high pneumonia incidence (37%) is probably due to sub-optimal environmental conditions within the calf house coupled with group housing of calves (Ballou, 2012). The pneumonia risk was further increased in the *ad libitum* MR fed calves both at group level and at individual calf level suggesting that group feeding *via* a single teat represented an additional risk. In addition, bed wetness was accentuated by the increased amounts of urine produced after consumption of large volumes of fluid in *ad libitum* MR fed calves. The association between communal feeding systems utilising a single teat and increased

incidence of pneumonia has been reported (Hepola, 2003; Maatje et al., 1993). Although since the use of such systems neccessitates group housing, a known risk factor for pneumonia, it is unclear whether the increased risk is associated with use of a shared teat *per se* or associated with group housing. The present study would unambiguously suggest that use of a shared teat is a risk factor in its own right. It may be hypothesised that this is associated with transfer of pathogens in saliva and nasal secretions from calf to calf *via* the teat. This is supported by studies demonstrating oral infection with *Mycoplasma* spp (Maunsell et al., 2012) and by a report of isolating pathogenic *Mycoplasma* spp from a shared teat on a farm with high levels of *Mycoplasma* associated pneumonia (Oultram, 2015).

Furthermore, there was a marked age difference in susceptibility to clinical pneumonia between dietary groups. The *ad libitum* MR fed calves were significantly older than restricted MR fed calves at the time of diagnosis (54 days *versus* 35 days). At first sight, this appeared counter-intuitive since the pathogen challenge was probably greater for *ad libitum* MR fed animals due to the shared teat. It may therefore be expected that these calves would succumb to disease at an earlier age than restricted MR fed animals. The association between immune response and plane of nutrition in the young calf is poorly understood with contradictory findings depending on which aspect of the immune response is scrutinised (Ballou, 2012; Ballou et al., 2015; Obeidat et al., 2013). However Ballou *et al* (2015) found an enhanced immune response to challenge with an oral *Salmonella enterica* var *typhimurium* vaccine in calves fed increased levels of MR during the pre-weaning period. This suggests that increased nutrition may be associated with an enhanced immune response. We may hypothesise that the dietary group age difference observed in the present study was associated with an increased resilience to pathogen challenge in *ad libitum* MR fed animals.

In this study, the lack of any impact of calf diarrhoea on growth during the pre-weaning and post-weaning periods may be attributed not only to prompt treatment with an oral fluid mixture but also on the practice of continuing MR feeding during therapy (Webb et al., 1969). The mortality rate amongst diseased calves in the current study was zero, reflecting the early detection and treatment of disease. Suprisingly, multivariable modelling suggested no impact of disease on growth during the pre-weaning period, although a history of

pneumonia during this period did impact on growth during the post-weaning period which is concerning. It has been recognised that pneumonia during the pre-weaning period may have a negative impact on both growth and performance in later life (Bach, 2011; van der Fels-Klerz et al., 2001; Waltner-Toews et al., 1986; Warnick et al., 1977).

Plasma total protein concentration at 48 hours of age had a significant positive impact on growth up to the time of conception, although this was not apparent during the first 12 weeks of life. The impact of PTP on long term growth was such that it remained an explanatory variable in the Cox regression model for time to first service. This association was further clarified by comparing animals from the upper and lower quartiles for age at conception, with animals in the upper quartile having significantly ($P < 0.05$) higher PTP concentrations (mean: 7.10 g/dL) compared to those in the bottom quartile (mean: 6.68 g/dL). These findings suggest that colostrum intake impacts not only on the immune status of the calf *via* transfer of immunoglobulins, but has other, at present poorly described, roles affecting future growth. Similar findings have been shown by other groups and it is likely that these findings illustrate a further mechanism by which early life colostrum intake has a long term impact on animal lifetime production (Faber et al., 2005; Van Amburgh et al., 2011).

Chapter 4 described a carcass dissection study of Holstein bull calves at birth, 3, 9 and 12 weeks of age. The primary objective was to describe the carcass composition of calves relative to age and nutritional status. Internal adipose tissue deposition was greater in the *ad libitum* MR fed calves at all ages. For restricted MR fed calves, not only was there less internal adipose tissue than *ad libitum* MR fed calves but there appeared to be less at 3 weeks of age compared to at birth. This suggests that internal adipose tissue was metabolised to provide energy for maintenance during the first three weeks of life, akin to the BCS loss observed during this period. Conversely, internal adipose depots increased in restricted MR fed calves between 9 and 12 weeks compared to the *ad libitum* MR fed calves – is this an epigenetic effect of the severe under nutrition experienced by these calves in the first three weeks of life? Although a greater internal adipose depostion in *ad libitum* MR fed calves was apparent, it is important to understand that this constituted only 10% of total adipose tissue. Carcass associated adipose tissue accounted for approximately 90% of total adipose deposition and there were no dietary group differences in this depot. Although

findings from the carcass dissection study were interesting, sample size was small and conclusions must be made with caution.

Spiral CT scanning technology was used for estimation of carcass muscle, adipose tissue and bone proportions and provided a quick and accurate method of carcass analysis (Kongsro et al., 2008). Since the completion of the study, a mobile CT scanning unit has become available. This would allow for the analysis of live animal composition under anaesthesia, thereby allowing carcass studies to be undertaken in live heifer calves. Although the apperture of the scanner would only allow the passage of animals up to approximately 12 weeks of age, it would provide further useful information on adipose deposition and mammary development in early life. The ability to perform body composition studies in live animals would allow for cohort studies to be undertaken linking early life composition with future health and production. This is particularly exciting in light of the central role that increased adiposity plays in post-partum disease (Drackley, 2011).

Glucose homeostasis and insulin sensitivity of a subset of heifer calves was assessed at 2, 12 and 39 weeks of age (Chapter 5). The hypothesis that dietary group was associated with insulin sensitivity was tested. In addition, the study set out to describe any changes in insulin sensitivity and glucose homeostasis with increasing age. The major finding of the study was that the insulin sensitivity of calves fed *ad libitum* MR during early life appeared to be unimpaired and efficiency of glucose metabolism was no different to that of calves fed restricted volumes of MR. Previous studies have reported the negative impact of feeding large volumes of MR (4 litres twice daily) on the insulin sensitivity of dairy calves (Bach et al., 2013) which may have a negative impact on adult health if it persisted until adulthood. However, the present study failed to show any negative impact on glucose homeostasis and insulin sensitivity when calves were fed increased volumes of MR *via* a true *ad libitum* feeding system. This suggests that increased volumes of MR can be safely fed to calves if fed in small volumes rather than in two large discrete feeds, although there were only 6 individual calves tested per dietary group. To confirm this finding, a greater number of animals should be studied.

Whilst the intervention was applied during the pre-weaning period, data collection continued until conception, as confirmed by ultrasound examination. In terms of body

weight, there was no clear evidence of any catch-up growth in the restricted MR fed group. Interestingly, differences in other morphometric measures between the 2 dietary groups disappeared over time. It is unclear why catch-up growth occurred with respect to morphometric measures (which included measures of skeletal growth) but not for body weight. One hypothesis is that the internal adipose tissue deposition in *ad libitum* MR fed calves was increased throughout the post-weaning period compared to restricted MR fed animals. Not only did BCS tend to be higher in the *ad libitum* fed cohort compared to their restricted fed counterparts throughout the study, but the carcass dissection study suggested greater internal adipose deposition occuring in the *ad libitum* fed calves prior to weaning. This could result in dietary group weight differences but not in measures of skeletal growth.

While the feeding regime for study animals during the pre-weaning and early post-weaning phases were highly controlled, this was not the case from 5 months of age onwards when heifers were fed variable quantities of 'left-over' Total Mixed Ration (TMR) from the adult lactating and dry cow diets. Sporadically, the diet was supplemented with maize and grass silages of varying quality. Overall, this practice of feeding left-overs with high starch content resulted in heifers becoming over-conditioned during the bulling period. This especially affected animals that failed to conceive to first service and remained in the group for longer. Although the benefits of appropriate growth during the pre-weaning period in *ad libitum* MR fed calves may have been diluted by sub-optimal post-weaning nutrition, animals from both dietary groups were subjected to this inappropriate diet and therefore no systematic bias was introduced.

One major Key Performance Indicator (KPI) associated with dairy heifer rearing is AFC; directly related to this is age at first service. Before the onset of the current study, timing of first service for heifers on the study farm was under discussion between farm staff and researchers. In order to reach a consensus on the correct time for first service, the peer reviewed and non-peer reviewed literature was searched and opinion was sought from dairy practitioners in the USA via the American Association of Bovine Practitioners Internet List. The consensus was that there were 2 targets to be met prior to first service. Threshold selected criteria for submission for first service were the attainment of a body weight of 380kg and a withers height of 125cm. These were the targets that were intended to be employed on the farm for the duration of the study. However, farm staff failed to utilise

weight and height data collected by the researcher for decision making regarding breeding. This likely had a deleterious impact on the achievement of optimal age at conception. When retrospectively evaluating final morphometric measures from study heifers it was interesting to find that only 40 out of the 100 heifers reached the 2 targets simultaneously. Generally, the height target was reached earlier than the body weight measure; survival curves presented in Chapter 6 (Figure 6.4) suggest there was up to 4 weeks difference between achievement of the 2 targets. This raises the question: which is the best KPI measure for timing of first service – body weight or withers height?

There were statistically non-significant trends for *ad libitum* MR fed animals to reach all reproductive KPIs (age at puberty, age at first service and age at conception) earlier than restricted MR fed animals. The *ad libitum* MR fed heifers achieved targets approximately 2 weeks earlier than their restricted MR fed counterparts, suggesting significant benefits to the increased feeding regime. Although not statistically significant, *ad libitum* MR fed heifers had a higher conception rate (57%) than restricted MR fed heifers (51%). The lack of statistical signifcance may be a reflection of the relatively small sample size (n = 50 per group). Although the *ad libitum* MR fed heifers performed better than the restricted MR fed animals, the target AFC of 24 months was not met by either group (predicted AFC: *ad libitum* MR fed: 25.3 months, restricted MR fed: 25.9 months). This may be due to farm management deficits with regards to oestrus detection, nutritional management and the failure to use available growth measures in this group of animals. It is well recognised that farmers often fail to appreciate the importance of correct breeding management of maiden heifers which results in significant financial loss. It is apparent that if the benefits of improved early life growth are to be realised, farm management practices must be optimised to ensure heifers are served as early as possible after becoming eligible. The target should be that all heifers are served within 21 days of achieving target weight or height. This requires both regular monitoring of height and/or body weight of heifers and active heat detection. A recent trend has been the development of hormonal interventions to allow fixed time insemination thereby eliminating the need for oestrus detection (Lima et al., 2013).

The *ad libitum* MR fed heifers reached all the measured reproductive KPIs at an earlier age than restricted MR fed animals. This in turn should have allowed for an earlier entry into

productive life for these animals. However, the cost of calf rearing up until entry into the milking herd was greater for *ad libitum* MR fed animals due to increased feeding costs during the first 12 weeks. Approximate costings for MR and concentrate feed for the 2 rearing strategies used during this study were £187/calf for *ad libitum* and £103/calf for restricted fed groups. The *ad libitum* MR feeding strategy cost £84 more per calf than the restricted strategy, this is the challenge that researchers are faced with when promoting increased milk or MR feeding to dairy farmers. However, the costs associated with delayed AFC are considerable, complex and farm specific. Factors to be considered include loss of potential milk production and increase in number of heifer replacements required e.g. if AFC is 24 months, 48 replacement heifers per 100 milking cows must be reared compared to 71 if AFC is 28 months (Kite, 2014). Thus it is apparent that productivity gains achieved with increased MR feeding and subsequent reduced AFC will considerably outweigh the additional costs of MR. In the current study, *ad libitum* MR fed heifers reached conception and therefore predicted first calving 2.3 weeks earlier than restricted MR fed heifers. According to current estimates, the financial implications of additional days not in calf (i.e. maintenance costs) are £2.30 per day (Laven, 2015). This demonstrates that approximately 50% of the additional cost of the *ad libitum* MR feeding strategy (£84) is made up for by earlier age to conception in *ad libitum* MR fed heifers.

In order for results from this study to be used to promote increased milk or MR feeding for dairy calves, the feeding strategy used must be cost effective. Chapter 3 of this thesis highlighted the importance of appropriate nutrition during the first 2 - 3 weeks of life. With this in mind, the *ad libitum* strategy could be refined to include a shorter *ad libitum* MR fed period. If *ad libitum* MR was fed for 3 - 4 weeks of life with a subsequent gradual weaning period of 3 weeks, the growth benefits may still be realised with a reduced financial investment. Whatever the length of the *ad libitum* MR fed period, the associated costs should be considered as an investment in the future of the dairy herd rather than an increase in rearing cost. Previous studies have reported the benefits of increased liquid milk feeding for at least the first 5 weeks of life on lifetime performance (Soberon et al., 2012; Soberon and Van Amburgh, 2013; Van Amburgh et al., 2011).

This study allowed for the careful monitoring and description of study calves reared on one farm from birth until conception. There were however, some limitations to the study. The intervention study was carried out on a dairy farm with 170 milking animals and a year round calving pattern. The time taken to recruit study animals was therefore rather lengthy. In fact, the pre-weaning phase of the study (the first 12 weeks) took 2 years to complete and there were often intervals of up to 2 weeks between calvings. If the number of calvings per year were greater, recruitment of calves and completion of the study would have been swifter. Alternatively, a greater number of calves could have been recruited within the same time period in order to add power to the study.

Another limitation of the study was the post-weaning diet of study heifers. In ideal circumstances, the diet of animals from 5 months of age until conception would be as closely regulated as it was for the first 5 months of life. This would allow for identification of the full benefits of *ad libitum* MR feeding during the pre-weaning period; however this study limitation is perhaps a reflection of real circumstances on many dairy farms.

This thesis forms the basis of a database of lifetime performance data of a cohort of 100 Holstein dairy heifers with relatively low genetic variation. Further work to determine the performance of these animals throughout their productive lives is crucial. The next stage would be to understand the short to medium term health and productivity of animals within the 2 dietary groups. Data to be evaluated would include first lactation milk yield, number of animals carrying their calves to term and fertility during first lactation. There are many studies that have documented the increase in first lactation milk yield of heifers fed increased milk or MR during the pre-weaned period (Bar-Peled et al., 1997; Bartlett et al., 2006; Drackley et al., 2007; Soberon et al., 2012; Van Amburgh et al., 2011). Although first lactation performance is an important factor, the lifetime performance of these animals and total production ability will allow cost-benefit analysis of the *ad libitum* MR feeding strategy. In turn, optimal rearing strategies in terms of animal welfare and future performance may be developed to allow for sustainable dairy farming across the U.K.

7.2 References

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Appendix A

Dairy Calf Questionnaire

Please tick boxes that apply or write in the spaces provided.

Farm Staff

1. How many people work on your farm in total?.....

Calf staff

2. Who looks after your pre-weaned calves? (tick all that apply)

You☐ Your spouse/partner☐ A member of staff ☐ Other☐ (Please state).....

3. a) What is the gender of staff who predominantly look after the pre-weaned calves?

male☐ female☐

b) How long have the people who predominantly look after the pre-weaned calves been doing so?

<1 year☐ 1-2 years☐ 3-5 years☐ 6-10 years☐ >10 years☐

c) What is the age of the people predominantly looking after the pre-weaned calves?

<20yrs☐ 20-30yrs☐ 31-40yrs☐ 41-50yrs☐ 51-60yrs☐ >60yrs☐

4. How many people in total look after the calves on a regular basis?

1☐ 2☐ 3☐ 4+☐

5. Does somebody different look after the calves at the weekend?

Yes☐ No☐ Sometimes

The Dairy Herd

6. Please state your total number of:-

Adult dairy animals..... Number between 12 months of age and calving.....

Number weaned to 12 months of age..... Number pre-weaning.....

7. What percentage of purchased animals are there in your herd?

None (0%)☐ % purchased (approx)..... All (100%)☐

8. What Breed/s are your animals? (tick all that apply)

Holstein Friesian☐ British Friesian☐ Channel Island (Jersey or Guernsey)☐

Other breed ☐ (please state) ..... Crossbred☐ (please state) .....

Milk yield

9. What is the average milk yield per cow per year (Litres)? .....

Fertility

10. What type of calving pattern do you have?

All year round☐ Spring☐ Autumn☐ Other☐ (please state).....

11. What is your Calving Index? (days).....

12. What is the cull rate (per year) of dairy cows? (%).....

13. What is the cull rate (per year) of your first calved heifers? (%).....

14. What type of Breeding policy do you use?

AI only☐ AI & Bull service☐ Bull service only☐

15. What Breeds of sire do you use
a) on adult cows?

Dairy only☐ Beef only☐ Both☐ state breed/s.....

b) on maiden heifers?

Dairy only☐ Beef only☐ Both☐ state breed/s.....

<u>Vaccination</u>

16. Do you vaccinate your

a) dairy herd? Yes☐ No☐ Sometimes☐

If yes/sometimes, what do you vaccinate for?

BVD☐ Leptospirosis☐ IBR☐ Rotavirus/Coronavirus/*E. coli*☐

Other☐ (specify) .....

b) pre-weaned calves? Yes☐ No☐ Sometimes☐

If yes/sometimes, what do you vaccinate for?

Pneumonia (including IBR)☐ Ringworm☐ Clostridial diseases☐

Other (specify).....

Management System

17. What best describes your system?

High input: High output☐ Medium input: Medium output☐ Low input: low output☐

18. Do you?

House milking cows all the year☐ House in winter, pasture in summer☐

House high yielders all year (low yielders outside in summer) ☐

Housing details

19. Where are the following animals housed:

a) Lactating cows?

Cubicles only☐ Straw yards only☐ Cubicles and yards☐ Other☐ (specify).....

b) Dry Cows?

Cubicles only☐ Straw yards only☐ Cubicles and yards☐ Other☐ (specify).....

c) Transition cows? (if housed separately)

Cubicles only☐ Straw yards only☐ Cubicles and yards☐

Other☐(specify).....

d) Growing stock (weaned to bulling)?

Cubicles only☐ Straw yards only☐ Cubicles and yards☐ Grazing in summer☐

Other☐ (specify).....

e) Bulling heifers?

Cubicles only☐ Straw yards only☐ Cubicles and yards☐

Other☐(specify).....

f) In calf heifers?

Cubicles only☐ Straw yards only☐ Cubicles and yards☐

Other☐(specify).....

Calving Management

20. Where do cows calve down:

a) In Summer?

All calve inside☐ Some calve inside & some calve outside☐ All calve outside☐

b) In Winter?

Individual calving boxes☐ Calving Group housing (cows at/around calving)☐

Dry cow (transition) housing☐ Other☐

21. If you don't have calving boxes, proceed to Q26

If you do have calving boxes:

a) How many calving boxes do you have?.....

b) What is the flooring in your calving box/es made of?

Concrete☐ Earth☐ Other☐ (Specify).....

c) What bedding is used?

Straw☐ Woodshavings☐ Sand ☐ None☐ Other☐

d) Do you put new bedding in for each cow? Yes☐ No ☐ Sometimes☐

e) How often is the box cleaned out completely?

After every calving☐ After every other calving☐ After 3-6 calvings☐

Every month☐ Every few months☐ Annually☐ Never☐

f) Do you ever disinfect the calving box/es? Yes☐ No☐

g) Do you use any sterilising compounds? (Lime, SOP etc). Yes☐ No☐

h) If yes, how often is this done?

After every calving☐ After every other calving☐ After 3-6 calvings☐

Every month☐ Every few months☐ Annually☐ Never☐

22. If you use only calving boxes, proceed to Q 27
If you calve cows in group housing or in dry cow accommodation

a) What is the flooring?

Concrete☐ Earth☐ Other☐ (Specify).....

b) What bedding is used?

Straw☐ Woodshavings☐ Sand ☐ None☐ Other☐

c) Do you use any sterilising compounds? (Lime, SOP etc). Yes☐ No☐

d) What is the average number of cows in the group?

2-10☐ 11-20☐ 21-30☐ 31+☐

e) How often do you bed down?

Daily☐ every 2-3 days☐ every 4-6 days☐ weekly ☐ Fortnightly☐

Monthly☐ Annually☐ Other☐ (Specify).....

f) How often do you clean the building out?

>Once/week☐ weekly ☐ Fortnightly ☐ Monthly ☐

Every 2-3 months☐ Annually☐ Other☐ (Specify).....

<u>Management of the newborn calf</u>

23. How long does the newborn calf stay with its dam?

Less than 3 hours☐ Less than 6 hrs☐ Less than 24 hours☐ 24 – 48 hours☐

2 – 4 days☐ More than 4 days☐

24. Do you dip the navel? Yes☐ No☐

25. What happens to Bull calves?

Disposed of at birth☐ Sold between 7 and 21 days old☐ Reared until weaning☐

Other☐ (please state).....

Colostrum management

26. Do you let the calf suckle the cow? Yes☐ No☐ Sometimes☐

27. To ensure the calf receives sufficient colostrum in the first day of life, do you?

Just allow natural suckling☐ Allow natural sucking & top up with extra if you think necessary☐

Feed all calves with colostrum☐

28. If you give colostrum to the calf, how do you give it?

Stomach tube/ calf feeder☐ Bucket and teat/bottle☐ Other☐ (Please specify) ……..

29. If you give colostrum, is it:

Usually from the mother if possible☐ Often from other cows☐ Usually from other cows or pooled☐

30. If you give colostrum, how long after birth do you feed it?

a) If the calf is born during the day:

Within 3 hours☐ within 6 hours☐ within 12 hours☐ within 24 hours☐

b) If the calf is born at night:

Within 3 hours☐ within 6 hours☐ within 12 hours☐ within 24 hours☐

31. Do you store frozen colostrum? Yes☐ No☐

Calf Housing

32. Once removed from the mother, where does the baby calf go?

Designated calf house☐ Shared building with weaned or older animals☐

Other☐ (please specify).....

33. Are pre-weaned calves kept in: (tick all that apply)

Individual pens☐ Group pens☐ Hutches☐ Other☐

(specify).....

34. If unweaned calves are group housed, at what age are they grouped?

Immediately (<24 hours)☐ 1day☐ 2days☐ 3– 7 days☐

8- 14 days☐ 15-28 days☐ >28 days☐ Not grouped☐

35. If calves are not group housed, proceed to question 40

If group housed:

a) how many calves per group?

2-4☐ 5-7☐ 8-10☐ 11+☐

b) What is the average maximum age difference of pre-weaned calves per group?

< 1 week☐ 1-2 weeks☐ 3-4 weeks☐ >4 weeks☐

36. What bedding material is used for calves?

Straw☐ Shavings☐ Sand☐ Other☐ (specify).....

37. How often is the calf/calves bedding cleaned out completely?

>Once/week☐ Once/week☐ every 2 weeks☐ Every month☐

Between calves/groups of calves☐ Every few months☐ Annually☐ Never☐

38. Do you ever disinfect the pens? Yes☐ No☐

If yes, how often is this done?

>Once/week☐ Once/week☐ every 2 weeks☐ Every month☐

Between calves/groups of calves☐ Every few months☐ Annually☐ Never☐

39. Do you use any sterilising compounds? (Lime, SOP etc). Yes☐ No☐

<u>Calf Feeding</u>

40. Which of the following are fed to baby calves? (tick all that apply)

Milk replacer ☐ Waste milk☐ Pooled colostrum☐

41. If milk replacer not used, proceed to Q46

a) Which brand and product of milk replacer do you use? (e.g. Volac Blossom Easymix)

Please specify.....

b) At what concentration is milk replacer made up?

100g/L☐ 125g/L☐ As per guidelines on bag ☐

Other ☐ Please Specify.....

c) How long prior to use is milk replacer made up?

Immediately☐ The morning of the day of use☐ A day before use☐

>1 day before use☐ Other☐ (specify).....

42. If whole milk (waste milk/pooled colostrum) is not used, proceed to Q47

a) If milk fed is whole milk, how long is it stored before use?

Fed straight away☐ up to 1 day☐ 2-4 days☐ 5-7 days☐ >1 week☐

b) How is this milk stored?

Refrigerated☐ Acidified☐ Other☐ (please specify).....

43. What temperature is milk/replacer fed at?

Chilled (4-8°C)☐ Room temperature☐ Warm☐ Body Temperature (37°C) ☐

Other☐ (specify).....

44. How are calves fed?

Individual bucket☐ Trough/milk bar☐ Automatic machine☐

Other☐ (please specify).....

45. Are preweaned calves fed ad libitum milk? Yes☐ No☐

46. If ad libitum milk fed, proceed to Q51

a) If not fed ad-libitum, how many times daily are calves fed?

Once☐ Twice☐ Three times☐ Don't know (machine fed)☐

Other☐

b) How much milk is fed at each feed (in litres)?

1-2☐ 2.1-3☐ 3.1-4☐ 4.1-5☐ Other☐(specify).....

47. Are calves fed from a:

Teat☐ Bucket☐ Other☐ (specify).....

48. How often are buckets or teat feeders washed?

Between feeds☐ Daily☐ Weekly☐ Never☐ Other☐
(specify).....

49. How often are buckets or teat feeders sterilised?

Between feeds☐ Daily☐ Weekly☐ Never☐ Other☐ (specify).....

50. Do calves have access to a dummy teat? Yes☐ No☐

51. If yes, do they use it? Always☐ Sometimes☐ Never☐

52. Do calves have an individual bucket/teat that is only used for them?

Yes☐ No☐

53. At what age is concentrate first offered to calves?

0 – 7 days☐ 8 – 14 days☐ After 2 weeks☐

54. What type of calf concentrate is fed before weaning?

Coarse mix☐ Pellet☐ Both☐ Other (specify)☐.....

55. Is concentrate fed ad libitum or restricted? ad libitum☐ restricted☐

56. At what age is forage first offered to calves?

0 – 7 days☐ 8 – 14 days☐ After 2 weeks☐

57. What type of forage is offered to calves before weaning?

Hay☐ Silage☐ Straw☐ Not offered but kept on straw bedding☐

None offered☐

58. a) Are calves given fresh water? Yes ☐ No ☐

b) Is water changed regularly? Yes ☐ No ☐

c) If yes, how often is water changed?

Daily☐ Every 2 days☐ Every week☐ Other☐

d) Is water available all the time? Yes ☐ No ☐

59. Do you give any routine medication to pre-weaned calves? Yes☐ **No**☐

60. If yes, what medication do you give?.....

61. Are calves routinely (tick all that apply):

Weighed☐ Girth measured (belly band)☐ Height measured (withers)☐ None of these☐

62. a) What criteria are used to decide when to wean calves? (tick all that apply)

Age☐ Concentrate intake☐ Body Size☐ Forage intake☐

Convenience /Management requirement e.g. to make room in housing☐

Other☐(specify).....

b) If concentrate intake is used to determine weaning, how much needed to be eaten per day for

weaning to occur? .....

63. What is the average age of weaning of your calves?.....

64. How are calves weaned? (Tick all that apply)

Abruptly☐ Gradual reduction in amount of milk☐ Gradual dilution of milk☐

Gradual increase in feeding interval☐

65. Are calves grouped for post weaning arrangements:

After weaning☐ On the same day as weaning☐

66. What type of calf concentrate is fed after weaning?

Coarse mix☐ Pellet☐ Both☐

Any other comments on your farm/ farming practices

.....
.....
.....
.....
.....

Thank you for your help

Appendix B

Table B.1: Gestation length, comparison of heifer and cow dams

Group	n	Mean gestation length (days)	95% CI	P Value
heifer	43	273.7	271.2 - 276.1	<0.001
cow	57	279.8	278.4 - 281.2	
combined	100	277.2	275.7 - 278.6	
difference		-6.14	-8.75 - 3.53	

Table B.2: Effect of date of birth on birth weight, with bull identity as random effect.

Outcome variable: birth weight			
	Coefficient	95% CI	P value
tsin4	0.659	0.393 -0.925	<0.001
tsin2	0.506	0.215 -0.798	0.001
tcos4	0.226	-0.032 -0.484	0.087
tcos2	-1.371	-1.682 --1.059	<0.001
dam parity	5.204	4.540 -5.868	<0.001
gestation	0.061	0.032 -0.090	<0.001
constant	21.690	13.737 -29.644	<0.001

Random-effects Parameters		
	Estimate	95% CI
bull ID	5.186	2.849 - 9.441
Residual	17.873	16.904 - 18.897

Table B.3: Effect of dam parity on birth weight.

Group	n	Mean birth weight (kg)	[95% Conf.	P Value
heifer	43	38.43	37.06 - 39.80	<0.001
cow	57	44.31	42.94 - 45.68	
combined	100	41.78	40.66 - 42.90	
difference		-5.88	-7.83 - 3.92	

Table B.4: Multivariable regression model including interaction terms and all variables affecting body weight during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: weight			
	Coefficient	**95% CI**	**P value**
1.adlib	2.006	-4.897 - 8.909	0.569
week 1	1.443	0.889 - 1.998	<0.001
week 2	1.844	0.744 - 2.944	0.001
week 3	3.947	2.712 - 5.182	<0.001
week 4	9.350	2.406 - 16.294	0.008
week 5	14.213	7.225 - 21.201	<0.001
week 6	19.825	12.785 - 26.866	<0.001
week 7	25.972	18.869 - 33.076	<0.001
week 8	32.348	25.173 - 39.523	<0.001
week 9	39.241	31.986 - 46.495	<0.001
week 10	46.110	38.767 - 53.452	<0.001
week 11	53.110	45.672 - 60.549	<0.001
week 12	61.606	54.064 - 69.147	<0.001
adlib#week 1	2.045	1.269 - 2.821	<0.001
adlib#week 2	3.635	2.092 - 5.178	<0.001
adlib#week 3	9.218	7.490 - 10.945	<0.001
adlib#week 4	9.203	2.121 - 16.285	0.011
adlib#week 5	10.426	3.260 - 17.591	0.004
adlib#week 6	11.769	4.502 - 19.036	0.002
adlib#week 7	12.446	5.062 - 19.831	0.001
adlib#week 8	13.261	5.744 - 20.779	0.001
adlib#week 9	14.160	6.496 - 21.824	<0.001
adlib#week 10	12.561	4.735 - 20.387	0.002
adlib#week 11	12.909	4.909 - 20.909	0.002
adlib#week 12	9.907	1.720 - 18.093	0.018
dam parity	6.132	4.196 - 8.069	<0.001
illness	-3.471	-5.929 - -1.013	0.006
age at 1st colostrum	-0.357	-0.793 - 0.079	0.109
temperature range	-0.083	-0.158 - -0.008	0.031
humidity range	-0.022	-0.045 - 0.002	0.074
min. temperature	0.131	0.061-0.201	<0.001
min. humidity	-0.033	-0.054 - -0.011	0.003
tsin4	-2.641	-3.277 - -2.005	<0.001
tsin2	1.681	0.526 - 2.836	0.004
tcos4	0.417	-0.272 - 1.107	0.236
tcos2	-0.392	-1.661 - 0.877	0.545
constant	43.872	36.675-51.069	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	10.235	3.194 - 32.794
calf		
variation	27.344	20.222 - 36.974
week	0.845	0.635 - 1.125
Residual	9.854	9.270 - 10.475

Table B.5: Multivariable regression model including interaction terms and all variables affecting withers height during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: withers height			
	Coefficient	95% CI	P value
ad libitum MR	-0.303	-1.467 - 0.860	0.610
week 1	1.418	0.869 - 1.967	<0.001
week 2	2.758	2.205 - 3.311	<0.001
week 3	3.913	3.366 - 4.460	<0.001
week 4	4.898	3.766 - 6.031	<0.001
week 5	6.338	5.198 - 7.478	<0.001
week 6	8.289	7.139 - 9.438	<0.001
week 7	9.882	8.722 - 11.042	<0.001
week 8	11.597	10.425 - 12.769	<0.001
week 9	13.379	12.194 - 14.564	<0.001
week 10	14.545	13.345 - 15.744	<0.001
week 11	16.198	14.984 - 17.413	<0.001
week 12	17.530	16.299 - 18.761	<0.001
adlib#week 1	0.509	-0.249 - 1.268	0.188
adlib#week 2	0.121	-0.653 - 0.895	0.759
adlib#week 3	1.437	0.671 - 2.202	<0.001
adlib#week 4	2.176	0.919 - 3.432	0.001
adlib#week 5	2.846	1.577 - 4.116	<0.001
adlib#week 6	2.585	1.301 - 3.869	<0.001
adlib#week 7	3.091	1.790 - 4.392	<0.001
adlib#week 8	2.6789	1.358 - 3.999	<0.001
adlib#week 9	2.986	1.642 - 4.330	<0.001
adlib#week 10	3.393	2.025 - 4.761	<0.001
adlib#week 11	3.499	2.106 - 4.891	<0.001
adlib#week 12	4.298	2.878 - 5.719	<0.001
dam parity	1.461	0.581 - 2.342	0.001
illness	-0.800	-1.862 - 0.261	0.139
volume of 1st colostrum	0.762	0.008 - 1.516	0.048
temperature range	0.049	0.013 - 0.085	0.007
tsin4	-0.054	-0.272 - 0.164	0.629
tsin2	0.431	0.078 - 0.784	0.017
tcos4	-0.133	-0.363 - 0.098	0.260
tcos2	-0.298	-0.680 - 0.084	0.127
constant	72.765	70.101 - 75.429	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	5.90 x10 $^{-13}$	0
calf		
variation	6.062	4.711 - 7.800
week	0.022	0.013 - 0.035
var(Residual)	1.687	1.546 - 1.841

Table B.6: Multivariable regression model including interaction terms and all variables affecting loin height during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: loin height			
	Coefficient	**95% CI**	**P value**
ad libitum MR	-0.217	-2.581 - 2.148	0.857
week 1	1.318	0.702 - 1.934	<0.001
week 2	2.465	1.844 - 3.085	<0.001
week 3	3.236	2.621 - 3.851	<0.001
week 4	4.821	2.485 - 7.157	<0.001
week 5	6.518	4.177 - 8.859	<0.001
week 6	8.003	5.656 - 10.349	<0.001
week 7	9.292	6.939 - 11.646	<0.001
week 8	11.312	8.951 - 13.673	<0.001
week 9	12.662	10.293 - 15.031	<0.001
week 10	13.910	11.531 - 16.289	<0.001
week 11	15.262	12.873 - 17.651	<0.001
week 12	16.413	14.013 - 18.812	<0.001
adlib#week 1	0.653	-0.199 - 1.505	0.133
adlib#week 2	0.654	-0.217 - 1.525	0.141
adlib#week 3	1.525	0.666 - 2.384	0.001
adlib#week 4	2.003	-0.413 - 4.418	0.104
adlib#week 5	2.182	-0.242 - 4.606	0.078
adlib#week 6	3.154	0.720 - 5.588	0.011
adlib#week 7	3.411	0.965 - 5.856	0.006
adlib#week 8	3.197	0.738 - 5.656	0.011
adlib#week 9	3.267	0.793 - 5.741	0.010
adlib#week 10	3.777	1.286 - 6.268	0.003
adlib#week 11	3.665	1.157 - 6.173	0.004
adlib#week 12	3.931	1.403 - 6.458	0.002
dam parity	1.351	0.429 - 2.273	0.004
illness	-0.749	-1.863 - 0.365	0.188
volume of 1st colostrum	1.003	0.208 - 1.797	0.013
age at 1st colostrum	-0.217	-0.413 - -0.020	0.030
temperature range	0.036	-0.010 - 0.083	0.126
min. humidity	-0.009	-0.020 - 0.002	0.105
tsin4	0.059	-0.188 - 0.306	0.640
tsin2	0.155	-0.256 - 0.566	0.459
tcos4	-0.151	-0.411 - 0.109	0.255
tcos2	-0.143	-0.581 - 0.294	0.520
constant	77.568	73.993 - 81.142	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	0.997	0.215 - 4.628
calf		
variation	5.930	4.437 - 7.927
week	0.027	0.017 - 0.043
Residual	2.127	1.947 - 2.325

Table B.7: Multivariable regression model including interaction terms and all variables affecting heart girth during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: heart girth			
	Coefficient	**95% CI**	**P value**
ad libitum MR	-0.361	-1.654 -0 .933	0.585
week 1	1.838	1.100 -2.576	<0.001
week 2	2.42	1.681 -3.156	<0.001
week 3	4.111	3.375 -4.847	<0.001
week 4	6.465	5.189 -7.742	<0.001
week 5	8.835	7.548 -10.122	<0.001
week 6	11.808	10.509 -13.107	<0.001
week 7	14.214	12.901 -15.527	<0.001
week 8	17.213	15.884 -18.542	<0.001
week 9	19.565	18.218 -20.912	<0.001
week 10	22.782	21.416 -24.148	<0.001
week 11	25.084	23.697 -26.470	<0.001
week 12	26.861	25.451 -28.270	<0.001
adlib#week 1	1.147	0.115 -2.179	0.029
adlib#week 2	2.018	0.972 -3.063	<0.001
adlib#week 3	4.111	3.071 -5.151	<0.001
adlib#week 4	5.467	3.988 -6.946	<0.001
adlib#week 5	6.294	4.799 -7.789	<0.001
adlib#week 6	5.982	4.467 -7.497	<0.001
adlib#week 7	7.222	5.684 -8.759	<0.001
adlib#week 8	6.843	5.280 -8.406	<0.001
adlib#week 9	6.934	5.342 -8.526	<0.001
adlib#week 10	5.957	4.333 -7.581	<0.001
adlib#week 11	6.138	4.480 -7.796	<0.001
adlib#week 12	6.255	4.561 -7.950	<0.001
dam parity	3.551	2.619 - 4.483	<0.001
plasma total protein	0.485	-0.071 - 1.042	0.087
illness	-1.655	-2.823 - -0.488	0.005
tsin4	0.214	-0.069 - 0.498	0.139
tsin2	-0.010	-0.448 - 0.428	0.965
tcos4	0.184	-0.112 - 0.481	0.223
tcos2	-0.383	-0.843 - 0.076	0.102
constant	77.981	73.975 - 81.986	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	5.00×10^{-11}	0
calf		
variation	6.671	5.115 – 8.700
week	0.033	0.020 – 0.056
Residual	3.374	3.095 – 3.679

Table B.8: Multivariable regression model including interaction terms and all variables affecting belly girth during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: belly girth			
	Coefficient	**95% CI**	**P value**
ad libitum MR	1.490	-2.257 - 5.238	0.436
week 1	2.592	0.835 - 4.348	0.004
week 2	3.804	2.047 - 5.561	<0.001
week 3	5.857	4.137 - 7.577	<0.001
week 4	10.101	6.389 - 13.812	<0.001
week 5	15.357	11.635 - 19.080	<0.001
week 6	19.810	16.074 - 23.546	<0.001
week 7	24.093	20.342 - 27.843	<0.001
week 8	28.887	25.117 - 32.656	<0.001
week 9	34.065	30.276 - 37.853	<0.001
week 10	38.371	34.560 - 42.181	<0.001
week 11	44.284	40.450 - 48.117	<0.001
week 12	47.629	43.770 - 51.487	<0.001
adlib#week 1	0.979	-1.437 - 3.396	0.427
adlib#week 2	3.731	1.281 - 6.181	0.003
adlib#week 3	6.870	4.468 - 9.272	<0.001
adlib#week 4	6.482	2.403 - 10.562	0.002
adlib#week 5	5.248	1.149 - 9.347	0.012
adlib#week 6	5.154	1.035 - 9.274	0.014
adlib#week 7	5.749	1.605 - 9.894	0.007
adlib#week 8	5.599	1.426 - 9.773	0.009
adlib#week 9	4.984	0.773 - 9.195	0.020
adlib#week 10	3.053	-1.194 - 7.299	0.159
adlib#week 11	1.047	-3.234 - 5.329	0.632
adlib#week 12	0.310	-4.013 - 4.634	0.888
dam parity	5.109	3.758 - 6.460	<0.001
plasma total protein	0.589	-0.201 - 1.380	0.144
illness	-3.093	-4.789 - -1.397	<0.001
volume of 1st colostrum	1.185	0.010 - 2.360	0.048
temperature range	0.138	0.026 - 0.249	0.015
tsin4	0.263	-0.296 - 0.823	0.356
tsin2	0.193	-0.596 - 0.983	0.631
tcos4	-0.400	-1.000 - 0.200	0.191
tcos2	-0.903	-1.772 - -0.035	0.042
constant	73.997	66.944 - 81.050	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	2.239	0.310 - 16.192
calf		
variation	9.315	6.173 - 14.057
week	0.098	0.053 - 0.179
Residual	16.782	15.377 - 18.315

Table B.9: Multivariable regression model including interaction terms and all variables affecting hock-fetlock length during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: hock-fetlock length			
	Coefficient	95% CI	P value
ad libitum MR	-0.228	-1.257 -0.802	0.664
week 1	1.058	0.605 - 1.510	<0.001
week 2	1.309	0.858 - 1.761	<0.001
week 3	1.501	1.059 - 1.943	<0.001
week 4	1.640	0.615 - 2.666	0.002
week 5	2.069	1.041 - 3.097	<0.001
week 6	2.360	1.330 - 3.391	<0.001
week 7	2.641	1.607 - 3.674	<0.001
week 8	3.228	2.191 - 4.265	<0.001
week 9	3.740	2.699 - 4.781	<0.001
week 10	4.247	3.202 - 5.292	<0.001
week 11	4.645	3.595 - 5.695	<0.001
week 12	4.967	3.912 - 6.022	<0.001
adlib#week 1	-0.400	-1.027 - 0.228	0.212
adlib#week 2	0.289	-0.345 - 0.923	0.371
adlib#week 3	0.213	-0.408 - 0.835	0.501
adlib#week 4	0.798	-0.317 - 1.914	0.161
adlib#week 5	0.992	-0.128 - 2.112	0.083
adlib#week 6	1.278	0.154 - 2.402	0.026
adlib#week 7	1.113	-0.015 - 2.242	0.053
adlib#week 8	1.300	0.165 - 2.434	0.025
adlib#week 9	1.240	0.098 - 2.382	0.033
adlib#week 10	1.055	-0.095 - 2.204	0.072
adlib#week 11	1.550	0.393 - 2.706	0.009
adlib#week 12	1.298	0.134 - 2.463	0.029
dam parity	0.486	0.142 - 0.829	0.006
volume of 1st colostrum	0.545	0.245 - 0.845	<0.001
temperature range	-0.038	-0.068 - -0.008	0.013
min. temperature	-0.057	-0.091 - -0.024	0.001
tsin4	-0.112	-0.256 - 0.032	0.127
tsin2	-0.285	-0.490 - -0.080	0.006
tcos4	0.477	0.324 - 0.630	<0.001
tcos2	-0.267	-0.530 - -0.004	0.047
constant	34.077	32.690 - 35.464	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	0.183	0.045 - 0.736
calf		
variation	0.640	0.434 - 0.941
week	0.005	0.003 - 0.102
Residual	1.164	1.067 - 1.270

Table B.10: Multivariable regression model including interaction terms and all variables affecting crown to rump length during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: crown to rump length			
	Coefficient	95% CI	P value
ad libitum MR	-0.181	-3.822 - 3.460	0.922
week 1	2.131	0.843 - 3.419	0.001
week 2	4.387	3.086 - 5.687	<0.001
week 3	5.911	4.615 - 7.206	<0.001
week 4	8.367	4.756 - 11.978	<0.001
week 5	10.190	6.560 - 13.820	<0.001
week 6	11.929	8.277 - 15.582	<0.001
week 7	14.467	10.789 - 18.146	<0.001
week 8	16.846	13.137 - 20.554	<0.001
week 9	19.713	15.971 - 23.455	<0.001
week 10	21.593	17.814 - 25.372	<0.001
week 11	24.372	20.554 - 28.190	<0.001
week 12	26.647	22.786 - 30.508	<0.001
adlib#week 1	0.413	-1.367 - 2.194	0.649
adlib#week 2	0.062	-1.767 - 1.890	0.947
adlib#week 3	0.975	-0.841 - 2.792	0.293
adlib#week 4	2.208	-1.634 - 6.050	0.260
adlib#week 5	4.374	0.498 - 8.249	0.027
adlib#week 6	6.032	2.118 - 9.946	0.003
adlib#week 7	5.351	1.390 - 9.312	0.008
adlib#week 8	6.012	1.999 - 10.025	0.003
adlib#week 9	5.594	1.521 - 9.666	0.007
adlib#week 10	7.129	2.991 - 11.266	0.001
adlib#week 11	6.618	2.414 - 10.823	0.002
adlib#week 12	6.476	2.198 - 10.755	0.003
dam parity	2.007	0.648 - 3.365	0.004
illness	-1.097	-2.778 - 0.583	0.201
volume of 1st colostrum	1.125	-0.056 - 2.307	0.062
temperature range	-0.084	-0.186 - 0.018	0.107
min. temperature	-0.096	-0.195 - 0.002	0.056
min. humidity	-0.026	-0.050 - -0.003	0.028
tsin4	0.607	0.107 - 1.106	0.017
tsin2	-0.112	-0.890 - 0.665	0.777
tcos4	0.097	-0.447 - 0.640	0.727
tcos2	1.313	0.376 - 2.251	0.006
constant	83.155	77.589 - 88.720	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	2.312	0.535 - 9.984
calf		
variation	11.065	7.911 - 15.477
week	0.175	0.118 - 0.261
Residual	9.292	8.502 - 10.155

Table B.11: Multivariable regression model including interaction terms and all variables affecting body condition score during the 0 to 12 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: body condition score			
	Coefficient	95% CI	P value
ad libitum MR	-0.022	-0.127 – 0.083	0.683
week 1	-0.162	-0.249 - -0.076	<0.001
week 2	-0.278	-0.365 - -0.191	<0.001
week 3	-0.315	-0.400 - -0.230	<0.001
week 4	-0.320	-0.422 - -0.219	<0.001
week 5	-0.172	-0.275 - -0.070	0.001
week 6	-0.077	-0.179 - 0.025	0.139
week 7	0.011	-0.090 - 0.113	0.827
week 8	-0.083	-0.186 - 0.019	0.110
week 9	0.019	-0.084 - 0.121	0.720
week 10	-0.023	-0.126 - 0.080	0.658
week 11	0.039	-0.064 - 0.143	0.459
week 12	0.080	-0.025 - 0.184	0.134
adlib#week 1	0.130	0.005 - 0.256	0.042
adlib#week 2	0.262	0.135 - 0.388	<0.001
adlib#week 3	0.433	0.309 - 0.558	<0.001
adlib#week 4	0.577	0.440 - 0.713	<0.001
adlib#week 5	0.543	0.406 - 0.679	<0.001
adlib#week 6	0.416	0.280 - 0.552	<0.001
adlib#week 7	0.395	0.260 - 0.531	<0.001
adlib#week 8	0.521	0.385 - 0.658	<0.001
adlib#week 9	0.501	0.363 - 0.638	<0.001
adlib#week 10	0.513	0.375 - 0.651	<0.001
adlib#week 11	0.392	0.253 - 0.531	<0.001
adlib#week 12	0.272	0.133 - 0.412	<0.001
dam parity	0.049	-0.007 - 0.104	0.084
plasma total protein	0.039	0.006 - 0.072	0.020
humidity range	0.002	0.001 - 0.003	0.007
min. temperature	-0.007	-0.013 - -0.001	0.020
tsin4	0.024	-0.002 - 0.049	0.069
tsin2	-0.041	-0.075 - -0.006	0.020
tcos4	-0.066	-0.092 - -0.041	<0.001
tcos2	0.018	-0.023 - 0.059	0.385
constant	2.452	2.201 - 2.702	<0.001

Random-effects Parameters		
	Estimate	95% CI
group		
variation	4.970×10^{-24}	1.210×10^{-45} - 0.020
calf		
variation	0.018	0.007 - 0.050
week	7.080×10^{-5}	1.310×10^{-5} - 3.818×10^{-4}
Residual	0.042	0.038 - 0.047

Table B.12: Effects of date of birth on the body weight of *ad libitum* MR fed calves.

Body weight	Coefficient	95% CI	P Value
week	6.067	5.990 -6.143	<0.001
dam parity	-1.430	-3.461 - 0.602	0.168
birth weight	1.191	1.012 - 1.369	<0.001
dobsin2	-0.527	-1.520 - 0.467	0.299
dobsin4	-1.093	-2.489 - 0.302	0.125
dobcos4	0.608	-0.728 - 1.943	0.372
dobcos2	3.685	2.214 - 5.156	<0.001
constant	-7.595	-14.514 - -0.675	0.031

Random-effects Parameters		
	Estimate	95% CI
calf		
variation	7.175	4.558 - 11.296
Residual	28.181	26.014 - 30.529

Table B.13: Effects of date of birth on the body weight of restricted MR fed calves.

weight	Coefficient	95% CI	P Value
week	4.672	4.592 - 4.752	0.000
dam parity	-0.297	- 1.917 - 1.323	0.719
birth weight	0.951	0.805 - 1.097	0.000
dobsin2	-0.508	- 1.431 - 0.415	0.281
dobsin4	-0.313	- 1.293 - 0.667	0.531
dobcos4	0.080	- 0.990 - 1.151	0.883
dobcos2	-1.063	- 2.193 - 0.066	0.065
constant	-0.635	- 6.474 - 5.204	0.831

Random-effects Parameters		
	Estimate	95% CI
calf		
variation	4.662	2.838 - 7.658
Residual	30.905	28.529 - 33.480

Appendix C

Table C.1: Individual organ weights (kg) recovered during dissection of bull calves in both Group A and R, studied at birth, 3, 9 and 12 weeks of age.

Age	Dietary group	Calf ID	Empty body mass	Spleen	Liver	Left kidney	Right kidney	Trachea and lungs	Heart	Thymus	Brain	Spinal cord	Urogenital tract	Testicles	Eyes
0		19	49.3	0.141	1.112	0.112	0.112	0.710	0.355	0.225	0.229	0.081	0.118	0.016	0.036
0		24	44.6	0.080	1.130	0.108	0.089	0.598	0.384	0.039	0.229	0.068	0.105	0.015	0.038
0		25	34.6	0.074	0.742	0.070	0.066	0.443	0.299	0.116	0.228	0.069	0.121	0.012	0.044
3	A	5	64.5	0.180	1.640	0.140	0.200	0.811	0.390	0.070	0.229	0.100	0.380	0.020	0.044
3	A	6	63.8	0.351	1.820	0.193	0.199	0.724	0.434	0.209	0.232	0.097	0.602	0.025	0.032
3	A	7	67.3	0.368	2.520	0.200	0.227	1.060	0.563	0.220	0.231	0.078	0.683	0.021	0.054
3	R	15	49.8	0.149	1.058	0.106	0.108	0.765	0.337	0.171	0.240	0.073	0.222	0.019	0.030
3	R	17	43.4	0.164	1.211	0.115	0.120	0.711	0.282	0.094	0.234	0.067	0.263	0.023	0.050
3	R	18	43.1	0.196	1.017	0.137	0.132	0.716	0.310	0.089	0.226	0.073	0.279	0.021	0.034
9	A	21	93.6	0.264	1.963	0.233	0.206	1.444	0.533	0.444	0.250	0.090	0.162	0.039	0.044
9	A	22	99.0	0.270	2.170	0.252	0.229	1.470	0.612	0.492	0.281	0.082	0.224	0.044	0.038
9	A	23	89.8	0.319	2.870	0.223	0.207	1.380	0.639	0.435	0.317	0.099	0.269	0.048	0.040
9	R	9	77.3	0.371	1.701	1.733	0.253	1.177	0.506	0.419	0.263	0.105	0.474	0.042	0.049
9	R	10	80.4	0.212	2.095	0.205	0.224	1.244	0.522	0.340	0.201	0.103	0.362	0.051	0.054
9	R	11	89.8	0.185	2.647	0.237	0.222	1.436	0.711	0.269	0.311	0.124	0.323	0.060	0.069
12	A	12	115.1	0.397	2.459	0.251	0.255	1.913	0.688	0.467	0.341	0.117	0.347	0.065	0.034
12	A	13	102.2	0.345	2.105	0.245	0.257	1.297	0.660	0.519	0.303	0.108	0.474	0.068	0.050
12	A	14	117.5	0.420	2.704	0.307	0.289	2.270	0.735	0.393	0.310	0.082	0.406	0.080	0.060
12	R	1	110.2	0.235	2.686	0.284	0.258	1.729	0.602	0.403	0.333	0.075	0.383	0.052	0.070
12	R	2	107.3	0.216	2.781	0.312	0.325	1.651	0.721	0.535	0.326	0.135	0.655	0.046	0.040
12	R	8	95.6	0.638	2.692	0.288	0.274	1.642	5.270	0.461	0.271	0.086	0.337	0.070	0.052

Table C.2: Weights of contents (kg) recovered from specific areas of gastro-intestinal tracts of bull calves in both Group A and R, studied at birth, 3, 9 and 12 weeks of age.

Age	Dietary group	Calf ID	Empty body mass	Abomasum contents	Small intestine contents	Colon contents	Caecum contents	Rectum contents	Rumen-reticulum contents	Faeces
0		19	49.3	0.004	0.091	0.076	0.019	0.000	0.008	0.000
0		24	44.6	1.080	0.310	0.059	0.000	0.340	0.114	0.000
0		25	34.6	0.008	0.061	0.018	0.040	0.291	0.028	0.000
3	A	5	64.5	1.070	1.290	0.070	0.070	0.000	1.510	0.000
3	A	6	63.8	2.380	1.270	0.073	0.140	0.000	0.170	0.170
3	A	7	67.3	1.670	1.920	0.072	0.066	0.023	2.600	0.312
3	R	15	49.8	0.290	0.804	0.158	0.464	0.000	0.976	0.000
3	R	17	43.4	2.359	0.578	0.173	0.091	0.027	2.395	0.000
3	R	18	43.1	0.865	0.680	0.183	0.101	0.145	1.394	0.000
9	A	21	93.6	1.570	3.200	0.457	0.288	0.000	6.270	0.100
9	A	22	99.0	0.720	2.090	0.610	0.111	0.000	4.962	0.000
9	A	23	89.8	3.170	1.700	0.282	0.166	0.000	4.570	0.300
9	R	9	77.3	1.257	2.304	0.561	0.193	0.000	7.730	0.170
9	R	10	80.4	1.595	2.689	0.406	0.480	0.000	14.730	0.190
9	R	11	89.8	1.856	2.990	1.035	0.449	0.000	13.370	0.000
12	A	12	115.1	2.708	3.190	0.640	0.512	0.000	13.350	0.525
12	A	13	102.2	2.720	2.300	0.585	0.991	0.000	8.930	0.300
12	A	14	117.5	0.867	4.310	0.992	0.079	0.000	12.780	0.483
12	R	1	110.2	0.916	3.450	0.681	0.403	0.124	11.558	0.193
12	R	2	107.3	1.131	4.550	1.223	0.345	0.2070	11.956	0.278
12	R	8	95.6	1.165	2.423	1.035	0.534	0.000	13.231	0.000

Table C.3: Weights of specific areas of gastro-intestinal tracts (kg) of bull calves in both Group A and R, studied at birth, 3, 9 and 12 weeks of age.

Age	Dietary group	Calf ID	Empty body mass	Oesophagus	Omasum	Rumen-reticulum	Abomasum	Small intestine	Rectum	Colon	Caecum
0		19	49.3	0.055	0.075	0.229	0.257	1.059	0.164	0.165	0.027
0		24	44.6	0.041	0.061	0.366	0.230	1.060	0.152	0.285	0.013
0		25	34.6	0.036	0.067	0.147	0.193	0.764	0.132	0.132	0.028
3	A	5	64.5	0.070	0.080	0.360	0.380	1.990	0.090	0.670	0.120
3	A	6	63.8	0.079	0.048	0.328	0.410	1.990	0.101	0.079	0.100
3	A	7	67.3	0.090	0.062	0.475	0.398	2.280	0.082	0.800	0.076
3	R	15	49.8	0.049	0.079	0.430	0.282	1.530	0.143	0.156	0.123
3	R	17	43.4	0.054	0.090	0.355	0.414	1.136	0.176	0.192	0.043
3	R	18	43.1	0.086	0.093	0.484	0.278	1.045	0.150	0.349	0.044
9	A	21	93.6	0.101	0.242	1.397	1.620	2.840	0.354	0.723	0.092
9	A	22	99.0	0.184	0.257	1.042	0.440	2.550	0.287	0.480	0.096
9	A	23	89.8	0.124	0.148	0.920	0.499	2.440	0.252	0.392	0.099
9	R	9	77.3	0.118	0.419	1.438	0.436	2.630	0.110	0.595	0.074
9	R	10	80.4	0.108	0.478	2.854	0.701	2.759	0.311	0.467	0.089
9	R	11	89.8	0.085	0.733	3.295	0.800	2.465	0.267	0.255	0.107
12	A	12	115.1	0.216	0.710	1.839	0.813	2.790	0.374	0.483	0.142
12	A	13	102.2	0.129	0.546	1.706	0.723	2.313	0.253	0.493	0.354
12	A	14	117.5	0.111	0.645	2.302	0.878	3.672	0.356	0.976	0.116
12	R	1	110.2	0.102	1.304	3.330	1.304	3.330	0.242	1.217	0.109
12	R	2	107.3	0.100	1.001	2.886	0.631	3.070	0.207	1.049	0.095
12	R	8	95.6	0.155	0.988	2.435	0.625	2.667	0.367	0.822	0.123

Table C.4: Weights of adipose tissue (kg) recovered during dissection of bull calves in both Group A and R, studied at birth, 3, 9 and 12 weeks of age.

Age	Dietary group	Calf ID	Empty body mass	Omental fat	Retroperitoneal fat	Cod fat	Intra-pelvic fat	Left kidney fat	Right kidney fat	Pericardial fat	Epicardial fat
0		19	49.3	0.000	0.102	0.054	0.032	0.181	0.126	0.026	0.059
0		24	44.6	0.000	0.000	0.060	0.041	0.081	0.077	0.013	0.009
0		25	34.6	0.000	0.000	0.020	0.150	0.068	0.080	0.013	0.018
3	A	5	64.5	0.090	0.140	0.040	0.170	0.100	0.060	0.000	0.020
3	A	6	63.8	0.104	0.096	0.072	0.026	0.133	0.181	0.000	0.116
3	A	7	67.3	0.045	0.174	0.050	0.273	0.190	0.235	0.038	0.071
3	R	15	49.8	0.083	0.046	0.012	0.014	0.050	0.073	0.017	0.007
3	R	17	43.4	0.000	0.018	0.002	0.016	0.052	0.056	0.015	0.019
3	R	18	43.1	0.000	0.060	0.018	0.012	0.079	0.097	0.021	0.048
9	A	21	93.6	0.373	0.194	0.150	0.395	0.208	0.196	0.034	0.058
9	A	22	99.0	0.000	0.306	0.212	0.416	0.218	0.201	0.043	0.066
9	A	23	89.8	0.000	0.438	0.128	0.429	0.223	0.239	0.056	0.042
9	R	9	77.3	0.000	0.000	0.092	0.073	0.150	0.139	0.037	0.025
9	R	10	80.4	0.000	0.056	0.073	0.086	0.097	0.102	0.032	0.038
9	R	11	89.8	0.000	0.182	0.080	0.200	0.175	0.158	0.170	0.028
12	A	12	115.1	0.000	0.270	0.230	0.384	0.290	0.303	0.075	0.101
12	A	13	102.2	0.894	0.360	0.186	0.358	0.190	0.160	0.046	0.043
12	A	14	117.5	0.416	0.302	0.096	0.536	0.191	0.287	0.087	0.057
12	R	1	110.2	0.528	0.000	0.146	0.147	0.142	0.145	0.000	0.043
12	R	2	107.3	0.000	0.134	0.176	0.222	0.317	0.373	0.172	0.056
12	R	8	95.6	0.000	0.282	0.120	0.252	0.121	0.212	0.037	0.037

Table C.5: Weight of Bone, skeletal muscle, adipose tissue (carcass associated and internal) and hide (kg) recovered during a combination of dissection and CT-analysis of bull calves in both Group A and R, studied at birth, 3, 9 and 12 weeks of age.

Age	Dietary group	Calf ID	Empty body mass	Bone	Skeletal muscle	Carcass associated adipose tissue	Internal adipose tissue	Hide
0		19	49.3	16.371	42.456	12.176	1.280	7.525
0		24	44.6	20.907	42.225	8.465	0.630	7.646
0		25	34.6	20.340	41.792	9.229	1.010	9.110
3	A	5	64.5	13.979	45.265	10.380	0.961	7.117
3	A	6	63.8	18.755	39.112	10.616	1.141	7.618
3	A	7	67.3	17.268	40.235	11.666	1.598	5.792
3	R	15	49.8	19.485	38.371	8.884	0.606	6.264
3	R	17	43.4	18.291	40.298	10.501	0.410	6.847
3	R	18	43.1	19.960	40.817	9.769	0.777	6.724
9	A	21	93.6	15.465	41.723	9.643	1.718	8.178
9	A	22	99.0	16.393	44.752	9.826	1.477	5.949
9	A	23	89.8	16.283	41.814	9.493	1.731	7.069
9	R	9	77.3	18.191	36.905	10.395	0.668	7.272
9	R	10	80.4	12.714	39.780	13.650	0.602	8.089
9	R	11	89.8	12.456	39.166	12.278	1.106	7.962
12	A	12	115.1	11.955	35.676	19.499	1.436	7.847
12	A	13	102.2	15.765	39.238	11.979	2.189	7.933
12	A	14	117.5	13.695	38.808	13.155	1.678	7.668
12	R	1	110.2	15.936	39.545	11.229	1.045	6.072
12	R	2	107.3	14.890	37.744	13.102	1.351	6.383
12	R	8	95.6	16.525	38.238	9.460	1.110	7.533

Appendix D

Table D.1: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma glucose concentration throughout the GTT for calves at 2 weeks of age. Calf is included as the random effect.

Outcome variable: Plasma glucose concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	-0.075	-0.600 - 0.450	0.780
1	-6.54×10^{-15}	-0.525 - 0.525	1.000
5	4.125	3.600 - 4.650	<0.001
10	3.092	2.566 - 3.617	<0.001
15	2.375	1.850 - 2.900	<0.001
25	1.825	1.300 - 2.350	<0.001
35	0.767	0.241 - 1.292	0.004
45	0.075	-0.450 - 0.600	0.780
60	-0.342	-0.867 - 0.184	0.202
75	-0.750	-1.275 - -0.225	0.005
90	-1.058	-1.583 - -0.533	<0.001
105	-1.075	-1.600 - -0.550	<0.001
120	-0.892	-1.417 - -0.367	0.001
135	-0.683	-1.209 - -0.158	0.011
150	-0.683	-1.209 - -0.158	0.011
ad libitum MR	-0.708	-1.233 - -0.183	0.008
time#*ad libitum* MR			
baseline#*ad lib*	-0.025	-0.768 - 0.718	0.947
1 #*ad lib*	-0.358	-1.101 - 0.385	0.345
5 #*ad lib*	-0.308	-1.051 - 0.435	0.416
10 #*ad lib*	-0.092	-0.835 - 0.651	0.809
15 #*ad lib*	-0.050	-0.793 0.693	0.895
25 #*ad lib*	0.008	-0.735 - 0.751	0.982
35 #*ad lib*	0.025	-0.718 - 0.768	0.947
45 #*ad lib*	0.233	-0.510 - 0.976	0.538
60 #*ad lib*	0.500	-0.243 - 1.243	0.187
75 #*ad lib*	0.833	0.090 - 1.576	0.028
90 #*ad lib*	0.708	-0.035 - 1.451	0.062
105 #*ad lib*	0.533	-0.210 - 1.276	0.159
120 #*ad lib*	0.383	-0.360 - 1.126	0.312
135 #*ad lib*	0.467	-0.276 - 1.210	0.218
150 #*ad lib*	0.392	-0.351 - 1.135	0.301
constant	5.5	4.932 - 6.068	<0.001

Table D.2: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma glucose concentration throughout the IST for calves at 2 weeks of age. Calf is included as the random effect.

Outcome variable: Plasma glucose concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	-0.083	-0.558 - 0.391	0.731
1	-0.008	-0.483 - 0.466	0.973
5	-0.908	-1.383 - -0.434	<0.001
10	-1.575	-2.049 - -1.101	<0.001
15	-2.175	-2.649 - -1.701	<0.001
25	-3.275	-3.749 - -2.801	<0.001
35	-3.550	-4.024 - -3.076	<0.001
45	-3.183	-3.658 - -2.709	<0.001
60	-2.792	-3.266 - -2.318	<0.001
75	-2.167	-2.641 - -1.693	<0.001
90	-1.833	-2.308 - -1.359	<0.001
105	-1.250	-1.724 - -0.776	<0.001
120	-0.992	-1.466 - -0.517	<0.001
135	-0.533	-1.008 - -0.059	0.027
150	-0.317	-0.791 - 0.158	0.191
ad libitum MR	0.383	-0.540 - 1.306	0.416
time#*ad libitum* MR			
baseline#*adlib*	-0.025	-0.696 - 0.646	0.942
1 #*adlib*	-0.133	-0.804 - 0.537	0.697
5 #*adlib*	0.267	-0.404 - 0.937	0.436
10 #*adlib*	0.025	-0.646 - 0.696	0.942
15 #*adlib*	-0.142	-0.812 - 0.529	0.679
25 #*adlib*	-0.117	-0.787 - 0.554	0.733
35 #*adlib*	-0.067	-0.737 - 0.604	0.846
45 #*adlib*	0.042	-0.629 - 0.712	0.903
60 #*adlib*	0.133	-0.537 - 0.804	0.697
75 #*adlib*	0.058	-0.612 - 0.729	0.865
90 #*adlib*	0.258	-0.412 - 0.929	0.450
105 #*adlib*	0.017	-0.654 - 0.687	0.961
120 #*adlib*	0.150	-0.521 - 0.821	0.661
135#*adlib*	-0.075	-0.746 - 0.596	0.826
150#*adlib*	-0.217	-0.887 - 0.454	0.527
constant	5.408	4.756 - 6.061	<0.001

Table D.3: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma glucose concentration throughout the CGIT for calves at 2 weeks of age. Calf is included as the random effect.

Outcome variable: Plasma glucose concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	0.258	-0.487 -1.004	0.497
1	3.850	3.105 -4.595	<0.001
5	2.517	1.771 -3.262	<0.001
10	1.358	0.613 -2.104	<0.001
15	0.250	-0.495 -0.995	0.511
25	-1.267	-2.012 --0.521	0.001
35	-2.250	-2.995 --1.505	<0.001
45	-2.600	-3.345 --1.855	<0.001
60	-2.350	-3.095 --1.605	<0.001
75	-2.108	-2.854 --1.363	<0.001
90	-1.883	-2.629 --1.138	<0.001
105	-1.600	-2.345 --0.855	<0.001
120	-1.375	-2.120 --0.630	<0.001
135	-0.917	-1.662 --0.171	0.016
150	-0.600	-1.345 -0.145	0.115
ad libitum MR	0.817	-0.353 - 1.986	0.171
time#*ad libitum* MR			
baseline#*ad lib*	-0.158	-1.213 -0.896	0.768
1 #*ad lib*	-0.167	-1.221 -0.888	0.757
5 #*ad lib*	-0.008	-1.063 -1.046	0.988
10 #*ad lib*	-0.173	-1.359 -1.013	0.775
15 #*ad lib*	-0.375	-1.429 -0.679	0.486
25 #*ad lib*	-0.600	-1.654 -0.454	0.265
35 #*ad lib*	-0.500	-1.554 -0.554	0.353
45 #*ad lib*	-0.367	-1.421 -0.688	0.495
60 #*ad lib*	0.375	-0.679 -1.429	0.486
75 #*ad lib*	0.667	-0.388 -1.721	0.215
90 #*ad lib*	0.725	-0.329 -1.779	0.178
105 #*ad lib*	0.883	-0.171 -1.938	0.101
120 #*ad lib*	0.883	-0.171 -1.936	0.101
135 #*ad lib*	0.650	-0.404 -1.704	0.227
150 #*ad lib*	0.417	-0.638 -1.471	0.439
constant	5.083	4.256 - 5.911	<0.001

Table D.4: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma NEFA concentration throughout the CGIT for calves at 2 weeks of age. Calf is included as the random effect.

Outcome variable: plasma NEFA concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	0.223	-0.119 - 0.565	0.200
1	0.222	-0.120 - 0.564	0.204
5	-0.072	-0.414 - 0.270	0.681
10	-0.315	-0.657 - 0.027	0.071
15	-0.393	-0.735 - -0.051	0.024
25	-0.423	-0.765 - -0.081	0.015
35	-0.353	-0.695 - -0.011	0.043
45	0.207	-0.135 - 0.539	0.236
60	0.440	0.098 - 0.782	0.012
75	0.552	0.210 - 0.894	0.002
90	0.480	0.138 - 0.822	0.006
105	0.483	0.141 - 0.825	0.006
120	0.323	-0.019 - 0.665	0.064
135	0.097	-0.245 - 0.439	0.579
150	0.210	-0.132 - 0.552	0.229
ad libitum MR	-0.017	-0.505 - 0.471	0.947
time#*ad libitum* MR			
baseline # *ad lib*	-0.195	-0.678 - 0.288	0.429
1 # *ad lib*	-0.018	-0.502 - 0.465	0.941
5 # *ad lib*	0.008	-0.475 - 0.492	0.973
10 # *ad lib*	0.307	-0.236 - 0.851	0.268
15 # *ad lib*	0.205	-0.278 - 0.688	0.406
25 # *ad lib*	0.230	-0.253 - 0.713	0.351
35 # *ad lib*	0.080	-0.403 - 0.563	0.746
45 # *ad lib*	-0.137	-0.620 - 0.347	0.580
60 # *ad lib*	0.032	-0.452 - 0.515	0.898
75 # *ad lib*	-0.343	-0.827 - 0.140	0.164
90 # *ad lib*	-0.253	-0.737 - 0.230	0.304
105 # *ad lib*	-0.333	-0.817 - 0.150	0.177
120 # *ad lib*	-0.020	-0.503 - 0.463	0.935
135 # *ad lib*	0.105	-0.378 - 0.588	0.670
150 # *ad lib*	0.067	-0.417 - 0.550	0.787
constant	0.538	0.193 - 0.883	0.002

Table D.5: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma insulin concentration throughout the CGIT for calves at 2 weeks of age. Calf is included as the random effect.

Outcome variable: plasma insulin concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
45	0.253	-1.642 - 2.149	0.793
75	-0.118	-2.014 - 1.778	0.903
ad libitum MR	-0.242	-2.596 - 2.113	0.841
time # *adlibitum* MR			
45 # *ad lib*	0.907	-1.775 - 3.588	0.507
75 # *ad lib*	2.265	-0.416 - 4.946	0.098
constant	0.418	-1.247 - 2.083	0.622

Table D.6: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma glucose concentration throughout the CGIT for calves at 12 weeks of age. Calf is included as the random effect.

Outcome variable: plasma glucose concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	-0.125	-0.626 - 0.376	0.625
1	5.342	4.840 - 5.843	<0.001
5	3.742	3.240 - 4.243	<0.001
10	2.583	2.082 - 3.085	<0.001
15	1.600	1.099 - 2.101	<0.001
25	0.333	-0.168 - 0.835	0.192
35	-0.408	-0.910 - 0.093	0.110
45	-0.817	-1.318 - -0.315	0.001
60	-0.992	-1.493 - -0.490	<0.001
75	-0.942	-1.443 - -0.440	<0.001
90	-0.975	-1.476 - -0.474	<0.001
105	-0.767	-1.268 - -0.265	0.003
120	-0.725	-1.226 - -0.224	0.005
135	-0.667	-1.168 - -0.165	0.009
150	-0.650	-1.151 - -0.149	0.011
1.adlib	0.0833333	-0.544 - 0.710	0.794
time# *ad libitum* MR			
baseline # *adlib*	0.0250001	-0.684 - 0.734	0.945
1 # *adlib*	0.3333333	-0.375 - 1.042	0.357
5 # *adlib*	0.283333	-0.425 - 0.992	0.433
10 # *adlib*	-0.150	-0.859 - 0.559	0.678
15 # *adlib*	0.1249999	-0.584 - 0.834	0.730
25 # *adlib*	-7.95×10^{-8}	-0.709 - 0.709	1.000
35 # *adlib*	3.97×10^{-8}	-0.709 - 0.709	1.000
45 # *adlib*	0.050	-0.659 - 0.759	0.890
60 # *adlib*	-0.0833334	-0.792 - 0.625	0.818
75 # *adlib*	-3.97×10^{-8}	-0.709 - 0.709	1.000
90 # *adlib*	0.175	-0.534 - 0.884	0.628
105 # *adlib*	0.0916667	-0.617 - 0.800	0.800
120 # *adlib*	0.2083333	-0.500 - 0.917	0.565
135 # *adlib*	0.2916666	-0.417 - 1.000	0.420
150# *adlib*	0.2749999	-0.434 - 0.984	0.447
constant	5.108333	4.665 - 5.552	<0.001

Table D.7: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma NEFA concentration throughout the CGIT for calves at 12 weeks of age. Calf is included as the random effect.

Outcome variable: plasma NEFA concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	-0.058	-0.395 - 0.278	0.734
1	-0.042	-0.378 - 0.295	0.808
5	-0.127	-0.463 - 0.210	0.461
10	-0.473	-0.810 - -0.137	0.006
15	-0.613	-0.950 - -0.277	<0.001
25	-0.803	-1.140 - -0.467	<0.001
35	-0.822	-1.158 - -0.485	<0.001
45	-0.608	-0.945 - -0.272	<0.001
60	-0.528	-0.865 - -0.192	0.002
75	-0.527	-0.863 - -0.190	0.002
90	-0.298	-0.635 - 0.038	0.082
105	-0.337	$-0.673 - -1.04 \times 10^{-7}$	0.050
120	-0.127	-0.463 - 0.210	0.461
135	-0.028	-0.365 - 0.308	0.869
150	0.035	-0.302 - 0.372	0.839
ad libitum MR	-0.285	-0.705 - 0.135	0.183
time#*adlib*			
5 1	0.372	-0.104 - 0.848	0.126
10 1	0.242	-0.234 - 0.718	0.320
11 1	-0.045	-0.521 - 0.431	0.853
15 1	0.128	-0.348 - 0.604	0.597
20 1	0.117	-0.359 - 0.593	0.631
25 1	0.095	-0.381 - 0.571	0.696
35 1	0.195	-0.281 - 0.671	0.422
45 1	0.288	-0.188 - 0.764	0.235
55 1	0.185	-0.291 - 0.661	0.446
70 1	0.258	-0.218 - 0.734	0.288
85 1	0.435	-0.041 - 0.911	0.073
100 1	0.230	-0.246 - 0.706	0.344
115 1	0.418	-0.058 - 0.894	0.085
130 1	0.405	-0.071 - 0.881	0.095
145 1	0.140	-0.336 - 0.616	0.564
160 1	-0.105	-0.581 - 0.371	0.666
constant	1.157	0.860 - 1.454	<0.001

Table D.8: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma insulin concentration throughout the CGIT for calves at 12 weeks of age. Calf is included as the random effect.

Outcome variable: plasma insulin concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
45	0.268	0.172 - 0.365	<0.001
75	-0.005	-0.101 - 0.091	0.919
ad libitum MR	0.003	-0.140 - 0.147	0.964
time # *adlib*			
45 # *adlib*	-0.085	-0.221 - 0.051	0.221
75 # *adlib*	-0.072	-0.208 - 0.065	0.302
constant	0.268	0.167 - 0.370	<0.001

Table D.9: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma glucose concentration throughout the CGIT for calves at 36 weeks of age. Calf is included as the random effect.

Outcome variable: plasma glucose concentration	**Coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	0.042	-0.714 - 0.797	0.914
1	7.850	7.094 - 8.606	0.000
5	4.817	4.061 - 5.572	0.000
10	3.258	2.503 - 4.014	0.000
15	2.100	1.344 - 2.856	0.000
25	0.575	-0.181 - 1.331	0.136
35	-0.475	-1.231 - 0.281	0.218
45	-1.167	-1.922 - -0.411	0.002
60	-1.450	-2.206 - -0.694	0.000
75	-1.100	-1.856 - -0.344	0.004
90	-0.933	-1.689 - -0.178	0.016
105	-0.708	-1.464 - 0.047	0.066
120	-0.542	-1.297- 0.214	0.160
135	-0.500	-1.256 - 0.256	0.195
150	-0.433	-1.189 - 0.322	0.261
ad libitum MR	0.133	-0.763 - 1.029	0.771
time#*adlib*			
baseline # *adlib*	-0.208	-1.277 - 0.861	0.702
1 # *adlib*	-0.400	-1.469 - 0.669	0.463
5 # *adlib*	0.717	-0.352 - 1.786	0.189
10 # *adlib*	-0.033	-1.102 - 1.036	0.951
15 # *adlib*	-0.333	-1.402 - 0.736	0.541
25 # *adlib*	-0.792	-1.861 - 0.277	0.147
35 # *adlib*	-0.683	-1.752 - 0.386	0.210
45 # *adlib*	-0.617	-1.686 - 0.452	0.258
60 # *adlib*	-0.150	-1.219 - 0.919	0.783
75 # *adlib*	0.125	-0.944 - 1.194	0.819
90 # *adlib*	0.333	-0.736 - 1.402	0.541
105 # *adlib*	0.267	-0.802 - 1.336	0.625
120 # *adlib*	0.292	-0.777 - 1.361	0.593
135 # *adlib*	0.300	-0.769 - 1.369	0.582
150 # *adlib*	0.392	-0.677 - 1.461	0.473
constant	4.425	3.792 - 5.058	0.000

Table D.10: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma NEFA concentration throughout the CGIT for calves at 36 weeks of age. Calf is included as the random effect.

Outcome variable: plasma NEFA concentration	**coefficient**	**95% CI**	**P value**
time (minutes)			
baseline	-0.008	-0.333 - 0.316	0.960
1	0.073	-0.251 - 0.398	0.658
5	-0.010	-0.335 - 0.315	0.952
10	-0.248	-0.573 - 0.076	0.134
15	-0.308	-0.633 - 0.016	0.063
25	-0.468	-0.793 - -0.144	0.005
35	-0.488	-0.813 - -0.164	0.003
45	-0.422	-0.746 - -0.097	0.011
60	-0.113	-0.438 - 0.211	0.494
75	0.045	-0.280 - 0.370	0.786
90	0.022	-0.303 - 0.346	0.896
105	0.127	-0.198 - 0.451	0.445
120	0.047	-0.278 - 0.371	0.778
135	0.078	-0.246 - 0.403	0.636
150	0.088	-0.236 - 0.413	0.594
ad libitum MR	0.137	-0.271 - 0.544	0.511
time # adlib			
baseline# adlib	-0.247	-0.706 - 0.213	0.292
1 # adlib	-0.283	-0.743 - 0.176	0.227
5# adlib	-0.295	-0.754 - 0.164	0.208
10# adlib	-0.135	-0.594 - 0.324	0.564
15# adlib	-0.205	-0.664 - 0.254	0.382
25# adlib	-0.197	-0.656 - 0.263	0.401
35# adlib	-0.167	-0.626 - 0.293	0.477
45# adlib	-0.188	-0.648 - 0.271	0.421
60# adlib	-0.247	-0.706 - 0.213	0.292
75# adlib	-0.348	-0.808 - 0.111	0.137
90# adlib	-0.393	-0.853 - 0.066	0.093
105# adlib	-0.427	-0.886 - 0.033	0.069
120# adlib	-0.243	-0.703 - 0.216	0.299
135# adlib	-0.285	-0.744 - 0.174	0.224
150# adlib	-0.297	-0.756 - 0.163	0.205
constant	0.665	0.377 - 0.953	<0.001

Table D.11: Random effects multivariable regression model (including an interaction term between dietary group and time during the test) to predict differences in plasma insulin concentration throughout the CGIT for calves at 36 weeks of age. Calf is included as the random effect.

Outcome variable: plasma insulin concentration	**coefficient**	**95% CI**	**P value**
time (minutes)			
45	0.488	0.241 - 0.736	<0.001
75	-0.040	-0.287 - 0.207	0.751
ad libitum MR	0.203	-0.180 - 0.587	0.299
time#*adlib*			
45	0.190	-0.160 - 0.540	0.287
75	-0.095	-0.445 - 0.255	0.594
constant	0.433	0.162 - 0.705	0.002

Appendix E

Table E.1: Mean average daily withers height change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average daily withers height change (cm) 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.184 (0.159 - 0.210, 49)	0.166 (0.141 - 0.190, 50)	0.142
16.00 - 19.99	0.207 (0.171 - 0.244, 49)	0.143 (0.113 - 0.173, 50)	0.008
20.00 - 23.99	0.136 (0.106 - 0.167, 49)	0.153 (0.127 - 0.179, 49)	0.405
24.00 - 27.99	0.127 (0.100 - 0.154, 49)	0.101 (0.080 - 0.122, 49)	0.127
28.00 - 31.99	0.107 (0.085 - 0.130, 49)	0.131 (0.107 - 0.155, 49)	0.072
32.00 - 35.99	0.128 (0.106 - 0.150, 49)	0.095 (0.073 - 0.117, 49)	0.036
36.00 - 39.99	0.114 (0.086 - 0.142, 49)	0.110 (0.084 - 0.136, 49)	0.582
40.00 - 43.99	0.089 (0.065 - 0.112, 49)	0.098 (0.072 - 0.125, 49)	0.302
44.00 - 47.99	0.101 (0.080 - 0.123, 49)	0.100 (0.079 - 0.122, 49)	0.525
48.00 - 51.99	0.071 (0.050 - 0.091, 49)	0.054 (0.031 - 0.077, 49)	0.289
52.00 - 55.99	0.058 (0.039 - 0.078, 49)	0.058 (0.035 - 0.080, 49)	0.048
56.00 - 59.99	0.043 (0.024 - 0.063, 49)	0.060 (0.037 - 0.083, 49)	0.141
60.00 - 63.99	0.056 (0.032 - 0.079, 46)	0.052 (0.026 - 0.077, 47)	0.584
64.99 - 67.99	0.072 (0.045 - 0.099, 38)	0.050 (0.024 - 0.076, 37)	0.125
68.00 - 71.99	0.062 (0.034 - 0.090, 28)	0.065 (0.034 - 0.097, 24)	0.430
72.00 - 75.99	0.067 (0.030 - 0.104, 18)	0.045 (-0.021 - 0.112, 12)	0.256
76.00 - 79.99	0.042 (0.006 - 0.078, 13)	0.010 (-0.068 - 0.087, 8)	0.169
80.00 - 83.99	0.038 (-0.018 - 0.095, 8)	0.059 (-0.066 - 0.184, 4)	0.323
84.00 - 87.99	0.054 (-0.010 - 0.118, 6)	0.014	
88.00 - 91.99	0.009 (-0.121 - 0.139, 4)	0.107	
92.00 - 95.99	0.073 (0.001 - 0.144, 3)	0.000	
96.00 -99.99	0.066 (-0.229 - 0.361, 2)	0.036	
100.00 - 103.99	0.016 (-0.733 - 0.765, 2)	0.054	
104.00 - 107.99	0.004	0	

Table E.2: Mean average daily loin height change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average daily loin height change (cm) 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.179 (0.152 - 0.207, 49)	0.212 (0.173 - 0.251, 50)	0.087
16.00 - 19.99	0.210 (0.175 - 0.246, 49)	0.157 (0.128 - 0.186, 50)	0.021
20.00 - 23.99	0.184 (0.158 - 0.201, 49)	0.146 (0.113 - 0.178, 49)	0.067
24.00 - 27.99	0.134 (0.117 - 0.151, 49)	0.122 (0.101 - 0.143, 49)	0.374
28.00 - 31.99	0.106 (0.084 - 0.127, 49)	0.144 (0.123 - 0.166, 49)	0.012
32.00 - 35.99	0.126 (0.106 - 0.145, 49)	0.095 (0.072 - 0.118, 49)	0.042
36.00 - 39.99	0.120 (0.100 - 0.140, 49)	0.111 (0.080 - 0.142, 49)	0.613
40.00 - 43.99	0.099 (0.080 - 0.118, 49)	0.084 (0.063 - 0.104, 49)	0.280
44.00 - 47.99	0.085 (0.059 - 0.111, 49)	0.082 (0.058 - 0.106, 49)	0.853
48.00 - 51.99	0.079 (0.055 - 0.103, 49)	0.080 (0.052 - 0.107, 49)	0.516
52.00 - 55.99	0.079 (0.056 - 0.102, 49)	0.059 (0.035 - 0.084, 49)	0.236
56.00 - 59.99	0.048 (0.028 - 0.068, 49)	0.059 (0.037 - 0.081, 49)	0.461
60.00 - 63.99	0.041 (0.021 - 0.061, 46)	0.046 (0.025 - 0.067, 47)	0.363
64.99 - 67.99	0.071 (0.044 - 0.098, 38)	0.061 (0.040 - 0.081, 37)	0.538
68.00 - 71.99	0.066 (0.033 - 0.100, 28)	0.048 (0.022 - 0.073, 24)	0.373
72.00 - 75.99	0.052 (0.008 - 0.096, 18)	0.044 (0.003 - 0.085, 12)	0.784
76.00 - 79.99	0.081 (0.046 - 0.115, 13)	0.034 (-0.037 - 0.105, 8)	0.144
80.00 - 83.99	0.017 (-0.044 - 0.079, 8)	0.022 (-0.008 - 0.053, 4)	0.550
84.00 - 87.99	0.064 (-0.005 - 0.133, 6)	-0.018 (1)	
88.00 - 91.99	0.014 (-0.138 - 0.167, 4)	0.107 (1)	
92.00 - 95.99	*0.029 (-0.025 - 0.083, 3)*	-0.086 (1)	
96.00 -99.99	0.100 (-0.898 - 1.098, 2)	0.139 (1)	
100.00 - 103.99	0.041 (-0.027 - 0.109, 2)	0.036 (1)	
104.00 - 107.99	0.007 (1)	0	

Table E.3: Mean average daily belly girth change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average daily belly girth change (cm) 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.560 (0.476 - 0.645, 49)	0.744 (0.686 - 0.801, 50)	<0.001
16.00 - 19.99	0.376 (0.306 - 0.437, 49)	0.372 (0.290 - 0.457, 50)	0.962
20.00 - 23.99	0.365 (0.288 - 0.443, 49)	0.303 (0.232 - 0.375, 49)	0.239
24.00 - 27.99	0.160 (0.097 - 0.224, 49)	0.168 (0.104 - 0.232, 49)	0.859
28.00 - 31.99	0.145 (0.082 - 0.208, 49)	0.137 (0.063 - 0.211, 49)	0.870
32.00 - 35.99	0.107 (0.052 - 0.163, 49)	0.172 (0.114 - 0.230, 49)	0.053
36.00 - 39.99	0.173 (0.115 - 0.231, 49)	0.147 (0.101 - 0.194, 49)	0.246
40.00 - 43.99	0.199 (0.126 - 0.271, 49)	0.199 (0.126 - 0.272, 49)	0.497
44.00 - 47.99	0.230 (0.091 - 0.368, 49)	0.081 (-0.070 - 0.232, 49)	0.074
48.00 - 51.99	0.095 (-0.045 - 0.236, 49)	0.211 (0.065 - 0.356, 49)	0.128
52.00 - 55.99	0.150 (0.066 - 0.234, 49)	0.184 (0.120 - 0.247, 49)	0.262
56.00 - 59.99	0.085 (-0.013 - 0.183, 49)	0.034 (-0.043 - 0.110, 49)	0.202
60.00 - 63.99	0.138 (0.057 - 0.219, 46)	0.201 (0.177 - 0.274, 47)	0.126
64.99 - 67.99	0.180 (0.115 - 0.246, 38)	0.129 (0.039 - 0.219, 37)	0.176
68.00 - 71.99	0.147 (0.020 - 0.274, 28)	0.174 (0.075 - 0.274, 24)	0.317
72.00 - 75.99	0.127 (0.040 - 0.214, 18)	0.182 (0.077 - 0.286, 12)	0.199
76.00 - 79.99	0.157 (0.048 - 0.265, 13)	0.049 (-0.153 - 0.252, 8)	0.129
80.00 - 83.99	0.147 (0.020 - 0.274, 8)	0.339 (-0.003 - 0.682, 4)	0.050
84.00 - 87.99	0.060 (-0.132 - 0.252, 6)	0.321 (1)	
88.00 - 91.99	0.179 (-0.029 - 0.386, 4)	-0.107 (1)	
92.00 - 95.99	*0.024 (-0,465 - 0.512, 3)*	0)1)	
96.00 -99.99	0.339 (-5.787 - 6.465, 2)	0.071 (1)	
100.00 - 103.99	-0.089 (-3.493 - 3.314, 2)	-0.036 (1)	
104.00 - 107.99	-0.071 (1)	0.321 (1)	

Table E.4: Mean average daily heart girth change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average heart girth change (cm) 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.341 (0.302 - 0.380, 49)	0.296 (0.252 - 0.341, 50)	0.066
16.00 - 19.99	0.269 (0.247 - 0.291, 49)	0.238 (0.187 - 0.288, 50)	0.132
20.00 - 23.99	0.231 (0.199 - 0.264, 49)	0.279 (0.207 - 0.351, 49)	0.112
24.00 - 27.99	0.186 (0.153 - 0.219, 49)	0.161 (0.130 - 0.192, 49)	0.135
28.00 - 31.99	0.178 (0.132 - 0.224, 49)	0.153 (0.088 - 0.218, 49)	0.260
32.00 - 35.99	0.173 (0.136 - 0.209, 49)	0.171 (0.134 - 0.207, 49)	0.530
36.00 - 39.99	0.199 (0.167 - 0.231, 49)	0.188 (0.163 - 0.213, 49)	0.303
40.00 - 43.99	0.182 (0.152 - 0.213, 49)	0.214 (0.191 - 0.236, 49)	0.103
44.00 - 47.99	0.179 (0.151 - 0.207, 49)	0.255 (0.100 - 0.410, 49)	0.165
48.00 - 51.99	0.169 (0.141 - 0.197, 49)	0.087 (-0.063 - 0.238, 49)	0.143
52.00 - 55.99	0.142 (0.047 - 0.237, 49)	0.153 (0.123 - 0.183, 49)	0.413
56.00 - 59.99	0.183 (0.075 - 0.291, 49)	0.126 (0.089 - 0.163, 49)	0.160
60.00 - 63.99	0.117 (0.063 - 0.171, 46)	0.144 (0.109 - 0.180, 47)	0.199
64.99 - 67.99	0.141 (0.104 - 0.178, 38)	0.190 (0.109 - 0.271, 37)	0.132
68.00 - 71.99	0.144 (0.103 - 0.185, 28)	0.088 (-0.010 - 0.186, 24)	0.127
72.00 - 75.99	0.147 (0.078 - 0.215, 18)	0.161 (0.121 - 0.200, 12)	0.373
76.00 - 79.99	0.104 (0.022 - 0.187, 13)	0.161 (0.026 - 0.295, 8)	0.201
80.00 - 83.99	0.121 (0.020 - 0.221, 8)	0.080 (-0.203 - 0.364, 4)	0.325
84.00 - 87.99	0.089 (0.008 - 0.171, 6)	0.250 (1)	
88.00 - 91.99	0.170 (-0.139 - 0.479, 4)	-0.071 (1)	
92.00 - 95.99	0.155 (0.052 - 0.257, 3)	0.071 (1)	
96.00 -99.99	0.107 (-1.254 - 1.469, 2)	0.179 (1)	
100.00 - 103.99	0.054 (-1.535 - 1.642, 2)	0.107 (1)	
104.00 - 107.99	0.321 (1)	0 (1)	

Table E.5: Mean average daily crown rump length change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average daily crown rump length change (cm) 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.202 (0.150 - 0.253, 49)	0.228 (0.170 - 0.286, 50)	0.251
16.00 - 19.99	0.233 (0.183 - 0.283, 49)	0.168 (0.115 - 0.221, 50)	0.074
20.00 - 23.99	0.246 (0.187 - 0.304, 49)	0.192 (0.143 - 0.240, 49)	0.079
24.00 - 27.99	0.259 (0.196 - 0.323, 49)	0.292 (0.234 - 0.350, 49)	0.222
28.00 - 31.99	0.152 (0.085 - 0.219, 49)	0.214 (0.167 - 0.262, 49)	0.067
32.00 - 35.99	0.229 (0.160 - 0.298, 49)	0.160 (0.113 - 0.207, 49)	0.053
36.00 - 39.99	0.186 (0.127 - 0.250, 49)	0.185 (0.141 - 0.229, 49)	0.536
40.00 - 43.99	0.175 (0.125 - 0.225, 49)	0.136 (0.087 - 0.185, 49)	0.135
44.00 - 47.99	0.153 (0.092 - 0.215, 49)	0.189 (0.124 - 0.254, 49)	0.212
48.00 - 51.99	0.111 (0.045 - 0.177, 49)	0.106 (0.036 - 0.175, 49)	0.458
52.00 - 55.99	0.211 (0.145 - 0.278, 49)	0.127 (0.061 - 0.193, 49)	0.036
56.00 - 59.99	0.118 (0.051 - 0.185, 49)	0.173 (0.107 - 0.240, 49)	0.120
60.00 - 63.99	0.115 (0.046 - 0.186, 46)	0.062 (0.000 - 0.125, 47)	0.128
64.00 - 67.99	0.132 (0.021 - 0.242, 38)	0.185 (0.114 - 0.257, 37)	0.207
68.00 - 71.99	0.088 (-0.015 - 0.191, 28)	0.065 (-0.036 - 0.166, 24)	0.375
72.00 - 75.99	0.063 (-0.083 - 0.210,18)	0.033 (-0.125 - 0.190, 12)	0.384
76.00 - 79.99	0.201 (0.071 - 0.330, 13)	0.228 (0.069 - 0.386, 8)	0.386
80.00 - 83.99	0.031 (-0.185 - 0.247, 8)	0.232 (-0.310 - 0.774, 4)	0.139
84.00 - 87.99	0.226 (0.020 - 0.432, 6)	0 (1)	
88.00 - 91.99	0.205 (-0.009 - 0.420, 4)	0 (1)	
92.00 - 95.99	-0.095 (-0.407 - 0.216, 3)	0.036 (1)	
96.00 -99.99	-0.071 (-2.794 - 2.651, 2)	0.036 (1)	
100.00 - 103.99	-0.071 (-1.433 - 1.290, 2)	0.179 (1)	
104.00 - 107.99	0.429 (1)	0.250 (1)	

Table E.6: Mean average daily hock-fetlock length change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average daily hock-fetlock length change (cm) 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.048 (0.032 - 0.064, 49)	0.048 (0.032 - 0.064, 50)	0.983
16.00 - 19.99	0.050 (0.036 - 0.065, 49)	0.032 (0.015 - 0.049, 50)	0.107
20.00 - 23.99	0.027 (0.008 - 0.046, 49)	0.052 (0.033 - 0.072, 49)	0.030
24.00 - 27.99	0.041 (0.025 - 0.057, 49)	0.023 (0.006 - 0.039, 49)	0.120
28.00 - 31.99	0.034 (0.017 - 0.050, 49)	-0.006 (-0.077 - 0.065, 49)	0.272
32.00 - 35.99	0.039 (0.024 - 0.054, 49)	0.080 (0.012 - 0.147, 49)	0.119
36.00 - 39.99	0.028 (0.016 - 0.040, 49)	0.034 (0.020 - 0.049, 49)	0.245
40.00 - 43.99	0.041 (0.028 - 0.055, 49)	0.018 (0.005 - 0.031, 49)	0.014
44.00 - 47.99	0.007 (-0.007 - 0.021, 49)	0.029 (0.014 - 0.044, 49)	0.015
48.00 - 51.99	0.032 (0.018 - 0.047, 49)	0.010 (-0.002 - 0.022, 49)	0.010
52.00 - 55.99	0.020 (0.007 - 0.033, 49)	0.016 (0.002 - 0.030, 49)	0.352
56.00 - 59.99	0.017 (0.005 - 0.030, 49)	0.020 (0.004 - 0.035, 49)	0.412
60.00 - 63.99	0.019 (0.006 - 0.027, 46)	0.011 (-0.003 - 0.026, 47)	0.229
64.00 - 67.99	0.010 (-0.006 - 0.027, 38)	0.019 (0.001 - 0.038, 37)	0.233
68.00 - 71.99	0.028 (0.004 - 0.052, 28)	0.022 (0.004 - 0.041, 24)	0.352
72.00 - 75.99	0.002 (-0.022 - 0.026, 18)	0.015 (-0.012 - 0.041, 12)	0.228
76.00 - 79.99	0.016 (-0.011 - 0.044, 13)	0.000 (-0.036 - 0.036, 8)	0.209
80.00 - 83.99	0.005 (-0.039 - 0.480, 8)	0.036 (-0.057 - 0.129, 4)	0.184
84.00 - 87.99	-0.018 (-0.088 - 0.052, 6)	0.000 (1)	
88.00 - 91.99	0.009 (-0.088 - 0.106, 4)	-0.036 (1)	
92.00 - 95.99	0.048 (-0.137 - 0.232, 3)	0.036 (1)	
96.00 -99.99	0.018 (-0.209 - 0.245, 2)	0.036 (1)	
100.00 - 103.99	-0.018 (-1.152 - 1.117, 2)	0.000 (1)	
104.00 - 107.99	0.071 (1)	0.036 (1)	

Table E.7: Mean average daily body condition score change for calves in both the *ad libitum* and restricted milk replacer fed groups at different time periods throughout the post-weaning period. Time periods are split into 4 week blocks.

Age (weeks)	**Mean average daily BCS change 95% CI**		**P Value**
	Restricted (95% CI, *n*)	***ad libitum* (95% CI, *n*)**	
12.00 - 15.99	0.004 (0.001 - 0.006, 49)	-0.001 (-0.003 - 0.001, 50)	0.001
16.00 - 19.99	-0.002 (-0.005 - 0.001, 49)	-0.002 (-0.004 - 0.001, 50)	0.391
20.00 - 23.99	-0.003 (-0.005 - -0.001, 49)	-0.002 (-0.004 - 0.001, 49)	0.206
24.00 - 27.99	0.004 (0.001 - 0.006, 49)	-0.003 (-0.006 - -0.001, 49)	<0.001
28.00 - 31.99	-0.004 (-0.007 - -0.002, 49)	0.002 (-0.001 - 0.005, 49)	0.002
32.00 - 35.99	0.001 (0.000 - 0.003, 49)	0.000 (-0.003 - 0.002, 49)	0.122
36.00 - 39.99	0.002 (0.000 - 0.004, 49)	0.002 (0.000 - 0.004, 49)	0.437
40.00 - 43.99	0.001 (-0.001 - 0.003, 49)	0.001 (-0.001 - 0.003, 49)	0.339
44.00 - 47.99	0.001 (-0.001 - 0.003, 49)	0.002 (-0.001 - 0.003, 49)	0.460
48.00 - 51.99	0.000 (-0.001 - 0.002, 49)	0.000 (-0.002 - 0.002, 49)	0.431
52.00 - 55.99	0.000 (-0.003 - 0.001, 49)	0.001 (-0.001 - 0.002, 49)	0.126
56.00 - 59.99	0.002 (0.000 - 0.004, 49)	0.000 (-0.002 - 0.001, 49)	0.054
60.00 - 63.99	0.002 (0.000 - 0.004, 46)	0.004 (0.001 - 0.007, 47)	0.134
64.00 - 67.99	0.003 (0.001 - 0.005, 38)	-0.001 (-0.003 - 0.001, 37)	0.002
68.00 - 71.99	-0.001 (-0.004 - 0.002, 28)	0.004 (0.001 - 0.007, 24)	0.007
72.00 - 75.99	0.001 (-0.002 - 0.003, 18)	0.001 (-0.004 - 0.005, 12)	0.447
76.00 - 79.99	0.004 (0.000 - 0.007, 13)	0.000 (-0.006 - 0.005, 8)	0.079
80.00 - 83.99	0.001 (-0.004 - 0.007, 8)	0.004 (-0.001 - 0.010, 4)	0.201
84.00 - 87.99	-0.002 (-0.012 - 0.087, 6)	-0.004 (1)	
88.00 - 91.99	0.005 (-0.006 - 0.016, 4)	-0.007 (1)	
92.00 - 95.99	0.001 (-0.004 - 0.006, 3)	0.007 (1)	
96.00 -99.99	0.014 (0.014 - 0.014, 2)	0.011 (1)	
100.00 - 103.99	-0.007 (-0.097 - 0.084, 2)	0.004 (1)	
104.00 - 107.99	0.011 (1)	0.011 (1)	

Table E.8: Individual regression analyses to assess variables that may have impacted on body weight. The equation included age (in weeks) in addition to the variable in question, but were unadjusted for other variables. Results of individual analyses are presented together in one table for ease.

Outcome variable: body weight	Coefficient	95% CI	P value
dam parity	1.545	0.608 - 2.481	0.001
week	5.705	5.616 - 5.793	<0.001
constant	44.844	40.125 - 49.564	<0.001
diarrhoea	6.457	2.909 - 10.004	<0.001
week	5.705	5.617 - 5.793	<0.001
constant	44.962	40.374 - 49.551	<0.001
pneumonia	-4.700	-8.343 - -1.057	0.011
week	5.703	5.615 - 5.791	<0.001
constant	50.476	46.037 - 54.914	<0.001
plasma TP	6.714	4.544 - 8.883	<0.001
week	5.662	5.572 - 5.752	<0.001
constant	3.794	-11.757 - 19.345	0.632
birth weight	2.484	2.194 - 2.773	<0.001
week	5.763	5.681 - 5.844	<0.001
constant	-56.964	-69.835 - -44.093	<0.001
dietary group	17.797	14.376 - 21.218	<0.001
week	5.727	5.642 - 5.813	<0.001
constant	38.988	34.551 - 43.426	<0.001

Table E.9: Multivariable regression model including interaction terms and all variables affecting body weight during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: Body weight	Coefficient	95 % CI	P value
ad libitum vs restricted MR	5.740	-0.842 - 12.322	0.087
week 1	1.696	-0.925 - 4.317	0.205
week 2	2.104	-3.138 - 7.346	0.431
week 3	4.271	-0.971 - 9.513	0.110
week 4	8.646	3.404 - 13.888	0.001
week 5	13.500	8.258 - 18.742	<0.001
week 6	19.333	14.091 - 24.575	<0.001
week 7	25.563	20.321 - 30.804	<0.001
week 8	31.750	26.508 - 36.992	<0.001
week 9	38.844	33.602 - 44.086	<0.001
week 10	45.490	40.248 - 50.731	<0.001
week 11	52.760	47.519 - 58.002	<0.001
week 12	61.229	55.987 - 66.471	<0.001
week 16	89.209	83.917 - 94.500	<0.001
week 20	115.615	110.373 - 120.857	<0.001
week 24	140.240	134.998 - 145.482	<0.001
week 28	159.125	153.883 - 164.367	<0.001
week 32	178.385	173.144 - 183.627	<0.001
week 36	197.500	192.258 - 202.742	<0.001
week 40	223.594	218.352 - 228.836	<0.001
week 44	248.479	243.237 - 253.721	<0.001
week 48	275.490	270.198 - 280.782	<0.001
week 52	298.596	293.304 - 303.888	<0.001
week 56	321.256	315.964 - 326.548	<0.001
week 60	339.745	334.453 - 345.037	<0.001
week 64	359.275	353.823 - 364.726	<0.001
week 68	379.833	373.859 - 385.808	<0.001
week 72	400.388	393.437 - 407.340	<0.001
week 76	414.525	406.017 - 423.033	<0.001
week 80	438.044	427.978 - 448.110	<0.001
week 84	465.737	453.469 - 478.005	<0.001
week 88	484.557	469.097 - 500.017	<0.001
week 92	522.049	502.143 - 541.954	<0.001
week 96	541.444	517.099 - 565.789	<0.001
week 100	599.312	564.936 - 633.689	<0.001
week 104	593.312	558.936 - 627.689	<0.001
week 108	657.458	623.065 - 691.851	<0.001
adlib#week 1	2.018	-1.669 - 5.704	0.283
adlib#week 2	3.795	-3.613 - 11.202	0.315
adlib#week 3	8.265	0.923 - 15.608	0.027
adlib#week 4	9.784	2.410 - 17.157	0.009
adlib#week 5	11.746	4.372 - 19.120	0.002
adlib#week 6	13.308	5.934 - 20.682	<0.001
adlib#week 7	14.329	6.987 - 21.672	<0.001
adlib#week 8	15.434	8.061 - 22.808	<0.001
adlib#week 9	16.177	8.804 - 23.551	<0.001
adlib#week 10	15.981	8.607 - 23.355	<0.001

adlib#week 11	16.067	8.693 - 23.441	<0.001
adlib#week 12	13.042	5.728 - 20.355	<0.001
adlib#week 16	16.813	9.403 - 24.222	<0.001
adlib#week 20	14.019	6.645 - 21.393	<0.001
adlib#week 24	14.810	7.402 - 22.218	<0.001
adlib#week 28	14.956	7.547 - 22.364	<0.001
adlib#week 32	14.081	6.672 - 21.489	<0.001
adlib#week 36	19.466	12.058 - 26.874	<0.001
adlib#week 40	18.810	11.402 - 26.218	<0.001
adlib#week 44	20.924	13.516 - 28.333	<0.001
adlib#week 48	21.497	14.053 - 28.941	<0.001
adlib#week 52	18.474	11.030 - 25.918	<0.001
adlib#week 56	19.960	12.517 - 27.404	<0.001
adlib#week 60	17.554	10.111 - 24.998	<0.001
adlib#week 64	25.735	18.106 - 33.364	<0.001
adlib#week 68	21.504	13.059 - 29.949	<0.001
adlib#week 72	20.962	10.944 -30.980	<0.001
adlib#week 76	32.762	19.585 - 45.940	<0.001
adlib#week 80	22.150	6.283 - 38.018	0.006
adlib#week 84	41.476	20.297 - 62.655	<0.001
adlib#week 88	98.901	61.193 - 136.609	<0.001
adlib#week 92	71.409	31.671 - 111.148	<0.001
adlib#week 96	60.014	17.877 - 102.152	0.005
adlib#week 100	32.146	-16.482 - 80.774	0.195
adlib#week 104	46.146	-2.482 - 94.774	0.063
dam parity	7.692	2.448 - 12.936	0.004
plasma tp	3.626	0.391 - 6.860	0.028
pneumonia	-8.431	-14.616 - -2.246	0.008
diarrhoea	-4.521	-10.184 - 1.142	0.118
constant	15.958	-6.6101 - 38.526	0.166

Random-effects Parameters (variance)	Estimate	95% CI
calf:	157.160	117.024 - 211.062
Bull:	20.556	2.046 - 206.530
Residual	300.417	287.161 - 314.286

Table E.10: Multivariable regression model including interaction terms and all variables affecting withers height during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: withers height	Coefficient	95% CI	P value
ad libitum vs restricted MR	-0.425	-2.044 - 1.195	0.607
week 1	1.156	0.345 - 1.967	0.005
week 2	2.393	1.583 - 3.202	<0.001
week 3	3.579	2.779 - 4.379	<0.001
week 4	4.595	3.795 - 5.395	<0.001
week 5	6.033	5.233 - 6.833	<0.001
week 6	7.956	7.156 - 8.756	<0.001
week 7	9.510	8.710 - 10.310	<0.001
week 8	11.316	10.516 - 12.116	<0.001
week 9	12.831	12.031 - 13.631	<0.001
week 10	14.143	13.343 - 14.943	<0.001
week 11	15.795	14.995 - 16.595	<0.001
week 12	17.041	16.241 - 17.841	<0.001
week 16	22.198	21.393 - 23.002	<0.001
week 20	28.016	27.216 - 28.816	<0.001
week 24	31.812	31.012 - 32.612	<0.001
week 28	35.316	34.516 - 36.116	<0.001
week 32	38.352	37.552 - 39.151	<0.001
week 36	41.945	41.145 - 42.745	<0.001
week 40	45.099	44.300 - 45.899	<0.001
week 44	47.608	46.808 - 48.408	<0.001
week 48	50.358	49.553 - 51.162	<0.001
week 52	52.287	51.483 - 53.092	<0.001
week 56	54.034	53.230 - 54.839	<0.001
week 60	55.200	54.396 - 56.005	<0.001
week 64	56.505	55.686 - 57.325	<0.001
week 68	58.842	57.972 - 59.712	<0.001
week 72	60.464	59.495 - 61.433	<0.001
week 76	61.924	60.792 - 63.056	<0.001
week 80	62.915	61.614 - 64.216	<0.001
week 84	65.358	63.810 - 66.906	<0.001
week 88	67.468	65.553 - 69.383	<0.001
week 92	67.779	65.347 - 70.211	<0.001
week 96	70.294	67.341 - 73.247	<0.001
week 100	70.759	66.620 - 74.898	<0.001
week 104	69.559	65.420 - 73.698	<0.001
week 108	68.344	64.200 - 72.489	<0.001
adlib#week 1	0.696	-0.447 - 1.840	0.232
adlib#week 2	0.407	-0.746 - 1.559	0.489
adlib#week 3	1.705	0.573 - 2.838	0.003
adlib#week 4	2.443	1.308 - 3.578	<0.001
adlib#week 5	3.109	1.974 - 4.245	<0.001
adlib#week 6	2.880	1.745 - 4.016	<0.001
adlib#week 7	3.464	2.332 - 4.597	<0.001
adlib#week 8	2.906	1.770 - 4.041	<0.001
adlib#week 9	3.420	2.284 - 4.555	<0.001
adlib#week 10	3.681	2.545 - 4.816	<0.001
adlib#week 11	3.822	2.687 - 4.958	<0.001

adlib#week 12	4.737	3.607 - 5.867	<0.001
adlib#week 16	4.122	2.983 - 5.261	<0.001
adlib#week 20	2.344	1.209 - 3.480	<0.001
adlib#week 24	2.673	1.534 - 3.811	<0.001
adlib#week 28	1.950	0.811 - 3.088	0.001
adlib#week 32	2.616	1.478 - 3.755	<0.001
adlib#week 36	1.671	0.532 - 2.809	0.004
adlib#week 40	1.654	0.515 - 2.792	0.004
adlib#week 44	1.866	0.728 - 3.005	0.001
adlib#week 48	1.929	0.787 - 3.071	0.001
adlib#week 52	1.501	0.360 - 2.643	0.010
adlib#week 56	1.361	0.219 - 2.503	0.020
adlib#week 60	1.897	0.755 - 3.039	0.001
adlib#week 64	2.124	0.964 - 3.283	<0.001
adlib#week 68	0.921	-0.317 - 2.158	0.145
adlib#week 72	1.192	-0.204 - 2.587	0.094
adlib#week 76	0.826	-0.905 - 2.557	0.350
adlib#week 80	0.045	-1.983 - 2.073	0.965
adlib#week 84	-1.183	-3.815 - 1.449	0.378
adlib#week 88	-4.623	-9.189 - -0.058	0.047
adlib#week 92	-1.934	-6.740 - 2.871	0.430
adlib#week 96	-4.449	-9.539 - 0.640	0.087
adlib#week 100	-3.915	-9.772 - 1.943	0.190
adlib#week 104	-1.215	-7.072 - 4.643	0.684
dam parity	0.773	-0.444 - 1.991	0.213
plasma tp	0.612	-0.139 - 1.362	0.110
pneumonia	-0.691	-2.126 - 0.745	0.346
diarrhoea	-0.810	-2.124 - 0.505	0.227
constant	72.074	66.824 - 77.325	<0.001

Random-effects Parameters	Estimate	95% CI
calf:	8.721	6.549 - 11.612
Bull:	0.727	0.076 - 6.974
Residual	4.255	4.030 - 4.492

Table E.11: Multivariable regression model including interaction terms and all variables affecting loin height during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: loin height	Coefficient	95% CI	P value
ad libitum vs restricted MR	0.205	-1.312 - 1.721	0.791
week 1	0.933	0.110 - 1.757	0.026
week 2	2.144	1.322 - 2.966	<0.001
week 3	2.914	2.102 - 3.726	<0.001
week 4	4.560	3.748 - 5.371	<0.001
week 5	6.270	5.458 - 7.082	<0.001
week 6	7.699	6.887 - 8.511	<0.001
week 7	8.951	8.139 - 9.763	<0.001
week 8	11.010	10.198 - 11.821	<0.001
week 9	12.349	11.537 - 13.161	<0.001
week 10	13.672	12.860 - 14.484	<0.001
week 11	15.039	14.227 - 15.851	<0.001
week 12	16.037	15.225 - 16.849	<0.001
week 16	21.078	20.262 - 21.895	<0.001
week 20	26.889	26.077 - 27.701	<0.001
week 24	32.032	31.221 - 32.844	<0.001
week 28	35.770	34.958 - 36.582	<0.001
week 32	38.737	37.925 - 39.549	<0.001
week 36	42.272	41.460 - 43.084	<0.001
week 40	45.585	44.773 - 46.396	<0.001
week 44	48.397	47.585 - 49.209	<0.001
week 48	50.684	49.867 - 51.501	<0.001
week 52	52.990	52.174 - 53.807	<0.001
week 56	55.046	54.229 - 55.862	<0.001
week 60	56.373	55.557 - 57.190	<0.001
week 64	57.470	56.639 - 58.302	<0.001
week 68	59.479	58.596 - 60.362	<0.001
week 72	61.190	60.207 - 62.174	<0.001
week 76	62.144	60.996 - 63.293	<0.001
week 80	64.503	63.182 - 65.823	<0.001
week 84	65.637	64.066 - 67.208	<0.001
week 88	67.630	65.686 - 69.574	<0.001
week 92	69.439	66.971 - 71.908	<0.001
week 96	69.592	66.595 - 72.589	<0.001
week 100	70.155	65.954 - 74.356	<0.001
week 104	71.455	67.254 - 75.656	<0.001
week 108	70.261	66.054 - 74.468	<0.001
adlib#week 1	0.832	-0.328 - 1.992	0.160

adlib#week 2	0.646	-0.523 - 1.816	0.279
adlib#week 3	1.617	0.468 - 2.767	0.006
adlib#week 4	2.043	0.891 - 3.195	0.001
adlib#week 5	2.175	1.023 - 3.328	<0.001
adlib#week 6	3.234	2.082 - 4.386	<0.001
adlib#week 7	3.596	2.447 - 4.746	<0.001
adlib#week 8	3.297	2.145 - 4.449	<0.001
adlib#week 9	3.290	2.138 - 4.442	<0.001
adlib#week 10	3.730	2.578 - 4.883	<0.001
adlib#week 11	3.629	2.477 - 4.781	<0.001
adlib#week 12	3.990	2.843 - 5.136	<0.001
adlib#week 16	4.887	3.732 - 6.043	<0.001
adlib#week 20	3.524	2.372 - 4.676	<0.001
adlib#week 24	2.321	1.166 - 3.477	<0.001
adlib#week 28	1.988	0.832 - 3.143	0.001
adlib#week 32	3.063	1.907 - 4.218	<0.001
adlib#week 36	2.227	1.072 - 3.383	<0.001
adlib#week 40	2.015	0.859 - 3.171	0.001
adlib#week 44	1.536	0.380 - 2.691	0.009
adlib#week 48	1.584	0.425 - 2.743	0.007
adlib#week 52	1.499	0.340 - 2.658	0.011
adlib#week 56	1.102	-0.057 - 2.261	0.062
adlib#week 60	1.401	0.242 - 2.560	0.018
adlib#week 64	1.575	0.398 - 2.751	0.009
adlib#week 68	1.282	0.025 - 2.538	0.046
adlib#week 72	0.983	-0.433 - 2.400	0.174
adlib#week 76	1.104	-0.653 - 2.861	0.218
adlib#week 80	-0.404	-2.463 - 1.654	0.700
adlib#week 84	-0.027	-2.699 - 2.644	0.984
adlib#week 88	-2.869	-7.503 - 1.765	0.225
adlib#week 92	-1.679	-6.556 - 3.199	0.500
adlib#week 96	-4.231	-9.396 - 0.934	0.108
adlib#week 100	-0.894	-6.839 - 5.051	0.768
adlib#week 104	-1.194	-7.139 - 4.751	0.694
dam parity	0.827	-0.284 - 1.937	0.145
plasma tp	0.756	0.071 - 1.441	0.031
pneumonia	-1.311	-2.621 - -0.001	0.050
diarrhoea	-1.290	-2.489 - -0.091	0.035
constant	75.526	70.730 - 80.323	<0.001

Random-effects Parameters	Estimate	95% CI
calf:	7.231	5.424 - 9.639
Bull:	1.73×10^{-7}	3.15×10^{-14} - 0.944
Residual	4.384	4.152 - 4.628

Table E.12: Multivariable regression model including interaction terms and all variables affecting heart girth during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: heart girth	Coefficient	95% CI	P value
ad libitum vs restricted MR	0.053	-2.335 - 2.441	0.965
week 1	1.590	-0.095 - 3.275	0.064
week 2	2.139	0.457 - 3.821	0.013
week 3	3.906	2.244 - 5.568	<0.001
week 4	6.260	4.598 - 7.922	<0.001
week 5	8.635	6.973 - 10.297	<0.001
week 6	11.614	9.952 - 13.276	<0.001
week 7	14.031	12.369 - 15.693	<0.001
week 8	17.041	15.380 - 18.703	<0.001
week 9	19.406	17.744 - 21.068	<0.001
week 10	22.635	20.973 - 24.297	<0.001
week 11	24.948	23.286 - 26.609	<0.001
week 12	26.739	25.077 - 28.401	<0.001
week 16	36.157	34.485 - 37.828	<0.001
week 20	43.718	42.057 - 45.380	<0.001
week 24	49.989	48.327 - 51.651	<0.001
week 28	55.448	53.786 - 57.109	<0.001
week 32	60.458	58.796 - 62.120	<0.001
week 36	65.260	63.598 - 66.922	<0.001
week 40	70.802	69.140 - 72.464	<0.001
week 44	75.802	74.140 - 77.464	<0.001
week 48	80.719	79.048 - 82.391	<0.001
week 52	85.421	83.750 - 87.093	<0.001
week 56	89.443	87.771 - 91.114	<0.001
week 60	94.570	92.899 - 96.242	<0.001
week 64	97.546	95.843 - 99.249	<0.001
week 68	100.937	99.129 - 102.744	<0.001
week 72	105.006	102.993 - 107.018	<0.001
week 76	108.567	106.216 - 110.918	<0.001
week 80	112.063	109.360 - 114.766	<0.001
week 84	117.922	114.707 - 121.137	<0.001
week 88	121.169	117.191 - 125.146	<0.001
week 92	126.965	121.914 - 132.016	<0.001
week 96	128.097	121.964 - 134.230	<0.001
week 100	134.240	125.644 - 142.836	<0.001
week 104	132.240	123.644 - 140.836	<0.001
week 108	148.940	140.332 - 157.548	<0.001
adlib#week 1	1.359	-1.016 - 3.733	0.262
adlib#week 2	2.172	-0.222 - 4.566	0.075

adlib#week 3	4.357	2.004 - 6.710	<0.001
adlib#week 4	5.627	3.269 - 7.986	<0.001
adlib#week 5	6.456	4.098 - 8.815	<0.001
adlib#week 6	6.151	3.792 - 8.509	<0.001
adlib#week 7	7.466	5.114 - 9.819	<0.001
adlib#week 8	7.030	4.671 - 9.388	<0.001
adlib#week 9	7.134	4.776 - 9.493	<0.001
adlib#week 10	6.170	3.812 - 8.529	<0.001
adlib#week 11	6.368	4.010 - 8.727	<0.001
adlib#week 12	6.419	4.072 - 8.766	<0.001
adlib#week 16	5.343	2.977 - 7.708	<0.001
adlib#week 20	4.455	2.096 - 6.813	<0.001
adlib#week 24	6.008	3.643 - 8.373	<0.001
adlib#week 28	4.998	2.632 - 7.363	<0.001
adlib#week 32	4.310	1.945 - 6.675	<0.001
adlib#week 36	4.362	1.997 - 6.728	<0.001
adlib#week 40	4.050	1.684 - 6.415	0.001
adlib#week 44	5.050	2.684 - 7.415	<0.001
adlib#week 48	7.341	4.968 - 9.713	<0.001
adlib#week 52	5.013	2.641 - 7.386	<0.001
adlib#week 56	5.326	2.953 - 7.698	<0.001
adlib#week 60	3.677	1.305 - 6.049	0.002
adlib#week 64	4.762	2.354 - 7.170	<0.001
adlib#week 68	6.068	3.497 - 8.640	<0.001
adlib#week 72	4.014	1.115 - 6.912	0.007
adlib#week 76	4.385	0.789 - 7.980	0.017
adlib#week 80	4.578	0.366 - 8.791	0.033
adlib#week 84	3.626	-1.839 - 9.092	0.193
adlib#week 88	10.771	1.289 - 20.254	0.026
adlib#week 92	2.975	-7.005 - 12.956	0.559
adlib#week 96	3.843	-6.727 - 14.412	0.476
adlib#week 100	2.700	-9.465 - 14.865	0.664
adlib#week 104	7.700	-4.465 - 19.865	0.215
dam parity	3.099	1.582 - 4.617	<0.001
plasma tp	0.681	-0.255 - 1.616	0.154
pneumonia	-2.065	-3.855 - -0.275	0.024
diarrhoea	-1.055	-2.694 - 0.585	0.207
constant	76.780	70.178 - 83.382	<0.001

Random-effects Parameters (variance)	Estimate	95% CI
calf:	13.133	9.771 - 17.650
Bull:	1.068	0.044 - 26.203
Residual	18.368	17.398 - 19.392

Table E.13: Multivariable regression model including interaction terms and all variables affecting belly girth during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: belly girth	Coefficient	95% CI	P value
ad libitum vs restricted MR	1.676	-1.676 - 5.027	0.327
week 1	1.790	-0.748 - 4.328	0.167
week 2	3.016	0.482 - 5.550	0.020
week 3	5.079	2.576 - 7.583	<0.001
week 4	9.225	6.722 - 11.729	<0.001
week 5	14.454	11.951 - 16.958	<0.001
week 6	18.954	16.451 - 21.458	<0.001
week 7	23.121	20.618 - 25.624	<0.001
week 8	28.163	25.659 - 30.666	<0.001
week 9	33.225	30.722 - 35.729	<0.001
week 10	37.392	34.889 - 39.895	<0.001
week 11	43.288	40.784 - 45.791	<0.001
week 12	46.829	44.326 - 49.333	<0.001
week 16	62.120	59.603 - 64.638	<0.001
week 20	73.059	70.555 - 75.562	<0.001
week 24	83.048	80.545 - 85.551	<0.001
week 28	87.621	85.118 - 90.124	<0.001
week 32	91.850	89.347 - 94.354	<0.001
week 36	94.767	92.264 - 97.270	<0.001
week 40	99.725	97.222 - 102.229	<0.001
week 44	104.996	102.493 - 107.499	<0.001
week 48	111.376	108.858 - 113.894	<0.001
week 52	113.929	111.411 - 116.447	<0.001
week 56	118.121	115.603 - 120.638	<0.001
week 60	120.355	117.837 - 122.872	<0.001
week 64	124.488	121.923 - 127.053	<0.001
week 68	128.735	126.012 - 131.458	<0.001
week 72	132.891	129.860 - 135.922	<0.001
week 76	135.990	132.449 - 139.531	<0.001
week 80	141.758	137.687 - 145.829	<0.001
week 84	145.760	140.918 - 150.603	<0.001
week 88	148.566	142.574 - 154.557	<0.001
week 92	155.394	147.786 - 163.001	<0.001
week 96	152.952	143.715 - 162.189	<0.001
week 100	178.085	165.139 - 191.032	<0.001
week 104	168.085	155.139 - 181.032	<0.001
week 108	175.936	162.972 - 188.901	<0.001
adlib#week 1	1.094	-2.483 - 4.671	0.549

adlib#week 2	3.774	0.168 - 7.381	0.040
adlib#week 3	6.934	3.390 - 10.478	<0.001
adlib#week 4	6.587	3.034 - 10.140	<0.001
adlib#week 5	5.307	1.754 - 8.860	0.003
adlib#week 6	5.195	1.642 - 8.748	0.004
adlib#week 7	5.938	2.394 - 9.482	0.001
adlib#week 8	5.517	1.964 - 9.070	0.002
adlib#week 9	4.822	1.269 - 8.375	0.008
adlib#week 10	3.063	-0.490 - 6.616	0.091
adlib#week 11	1.208	-2.345 - 4.761	0.505
adlib#week 12	0.283	-3.252 - 3.819	0.875
adlib#week 16	5.763	2.200 - 9.326	0.002
adlib#week 20	5.417	1.864 - 8.970	0.003
adlib#week 24	4.058	0.495 - 7.621	0.026
adlib#week 28	4.048	0.485 - 7.611	0.026
adlib#week 32	3.756	0.193 - 7.319	0.039
adlib#week 36	5.756	2.193 - 9.319	0.002
adlib#week 40	5.048	1.485 - 8.611	0.005
adlib#week 44	5.048	1.485 - 8.611	0.005
adlib#week 48	1.106	-2.467 - 4.679	0.544
adlib#week 52	4.365	0.792 - 7.938	0.017
adlib#week 56	5.361	1.788 - 8.934	0.003
adlib#week 60	3.981	0.408 - 7.555	0.029
adlib#week 64	5.581	1.954 - 9.208	0.003
adlib#week 68	3.764	-0.109 - 7.637	0.057
adlib#week 72	3.901	-0.465 - 8.268	0.080
adlib#week 76	5.804	0.389 - 11.220	0.036
adlib#week 80	0.568	-5.776 - 6.913	0.861
adlib#week 84	8.711	0.479 - 16.943	0.038
adlib#week 88	20.371	6.089 - 34.653	0.005
adlib#week 92	10.543	-4.489 - 25.574	0.169
adlib#week 96	12.984	-2.934 - 28.903	0.110
adlib#week 100	-10.149	-28.471 - 8.173	0.278
adlib#week 104	-1.149	-19.471 - 17.173	0.902
dam parity	4.348	2.353 - 6.344	<0.001
plasma tp	1.342	0.112 - 2.572	0.033
pneumonia	-2.597	-4.951 - -0.244	0.031
diarrhoea	-2.182	-4.338 - -0.027	0.047
constant	74.485	65.770 - 83.200	<0.001

Random-effects Parameters (variance)	Estimate	95% CI
calf:	22.335	16.537 - 30.165
Bull:	4.924	1.231 - 19.697
Residual	41.681	39.480 - 44.004

Table E.14: Multivariable regression model including interaction terms and all variables affecting crown to rump length during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: crl	Coefficient	95% CI	P value
ad libitum vs restricted MR	0.773	-2.134 - 3.680	0.602
week 1	2.111	-0.051 - 4.273	0.056
week 2	4.374	2.216 - 6.533	<0.001
week 3	5.960	3.828 - 8.093	<0.001
week 4	8.002	5.870 - 10.134	<0.001
week 5	9.856	7.724 - 11.989	<0.001
week 6	11.648	9.515 - 13.780	<0.001
week 7	14.106	11.974 - 16.239	<0.001
week 8	16.648	14.515 - 18.780	<0.001
week 9	19.242	17.109 - 21.374	<0.001
week 10	21.148	19.015 - 23.280	<0.001
week 11	24.044	21.911 - 26.176	<0.001
week 12	26.377	24.245 - 28.509	<0.001
week 16	32.054	29.910 - 34.199	<0.001
week 20	38.398	36.265 - 40.530	<0.001
week 24	45.085	42.953 - 47.218	<0.001
week 28	52.419	50.286 - 54.551	<0.001
week 32	56.814	54.682 - 58.947	<0.001
week 36	63.189	61.057 - 65.322	<0.001
week 40	67.981	65.849 - 70.114	<0.001
week 44	73.127	70.995 - 75.259	<0.001
week 48	77.293	75.148 - 79.438	<0.001
week 52	80.527	78.382 - 82.672	<0.001
week 56	86.463	84.318 - 88.608	<0.001
week 60	89.888	87.744 - 92.033	<0.001
week 64	92.543	90.359 - 94.728	<0.001
week 68	96.525	94.205 - 98.845	<0.001
week 72	99.640	97.058 - 102.222	<0.001
week 76	101.233	98.216 - 104.250	<0.001
week 80	105.425	101.957 - 108.893	<0.001
week 84	105.300	101.175 - 109.425	<0.001
week 88	109.643	104.539 - 114.746	<0.001
week 92	117.153	110.672 - 123.633	<0.001
week 96	111.618	103.748 - 119.487	<0.001
week 100	119.336	108.306 - 130.365	<0.001
week 104	114.336	103.306 - 125.365	<0.001
week 108	131.035	119.991 - 142.079	<0.001
adlib#week 1	0.494	-2.553 - 3.541	0.751
adlib#week 2	-0.207	-3.279 - 2.865	0.895

adlib#week 3	0.909	-2.110 - 3.928	0.555
adlib#week 4	2.399	-0.627 - 5.426	0.120
adlib#week 5	4.463	1.437 - 7.490	0.004
adlib#week 6	6.141	3.114 - 9.168	<0.001
adlib#week 7	5.801	2.782 - 8.820	<0.001
adlib#week 8	6.202	3.176 - 9.229	<0.001
adlib#week 9	6.017	2.990 - 9.043	<0.001
adlib#week 10	7.621	4.594 - 10.647	<0.001
adlib#week 11	7.031	4.004 - 10.058	<0.001
adlib#week 12	6.497	3.484 - 9.509	<0.001
adlib#week 16	7.449	4.413 - 10.484	<0.001
adlib#week 20	5.881	2.854 - 8.907	<0.001
adlib#week 24	4.243	1.208 - 7.278	0.006
adlib#week 28	5.264	2.229 - 8.299	0.001
adlib#week 32	6.931	3.895 - 9.966	<0.001
adlib#week 36	4.847	1.812 - 7.883	0.002
adlib#week 40	5.222	2.187 - 8.258	0.001
adlib#week 44	3.931	0.895 - 6.966	0.011
adlib#week 48	5.182	2.138 - 8.226	0.001
adlib#week 52	4.989	1.945 - 8.033	0.001
adlib#week 56	2.407	-0.637 - 5.451	0.121
adlib#week 60	3.815	0.771 - 6.859	0.014
adlib#week 64	3.176	0.086 - 6.266	0.044
adlib#week 68	4.013	0.713 - 7.312	0.017
adlib#week 72	3.367	-0.353 - 7.086	0.076
adlib#week 76	0.408	-4.206 - 5.021	0.862
adlib#week 80	3.114	-2.291 - 8.519	0.259
adlib#week 84	5.349	-1.664 - 12.362	0.135
adlib#week 88	7.393	-4.774 - 19.559	0.234
adlib#week 92	-0.117	-12.923 - 12.688	0.986
adlib#week 96	6.418	-7.144 - 19.979	0.354
adlib#week 100	-0.301	-15.909 - 15.308	0.970
adlib#week 104	9.699	-5.909 - 25.308	0.223
dam parity	1.573	-0.190 - 3.336	0.080
plasma tp	0.837	-0.250 - 1.924	0.131
pneumonia	-2.157	-4.237 - -0.078	0.042
diarrhoea	-2.463	-4.367 - -0.558	0.011
constant	78.959	71.268 - 86.650	<0.001

Random-effects Parameters (variance)	Estimate	95% CI
calf:	17.520	12.995 - 23.621
Bull:	4.06×10^{-10}	2.95×10^{-17} - 0.006
Residual	30.246	28.649 - 31.932

Table E.15: Multivariable regression model including interaction terms and all variables affecting hock-fetlock length during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: hock-fetlock length	Coefficient	95% CI	P value
ad libitum vs restricted MR	-0.334	-0.960 - 0.291	0.295
week 1	0.850	0.388 -1.312	<0.001
week 2	1.161	0.699 - 1.622	<0.001
week 3	1.493	1.037 - 1.949	<0.001
week 4	1.629	1.172 - 2.085	<0.001
week 5	2.066	1.610 - 2.522	<0.001
week 6	2.379	1.922 - 2.83	<0.001
week 7	2.660	2.204 - 3.116	<0.001
week 8	3.274	2.818 - 3.730	<0.001
week 9	3.774	3.318 - 4.230	<0.001
week 10	4.295	3.839 - 4.751	<0.001
week 11	4.712	4.256 - 5.168	<0.001
week 12	5.087	4.631 - 5.543	<0.001
week 16	6.415	5.957 - 6.874	<0.001
week 20	7.795	7.339 - 8.251	<0.001
week 24	8.649	8.193 - 9.105	<0.001
week 28	9.712	9.256 - 10.168	<0.001
week 32	10.629	10.172 - 11.085	<0.001
week 36	11.754	11.297 - 12.210	<0.001
week 40	12.524	12.068 - 12.980	<0.001
week 44	13.691	13.235 - 14.147	<0.001
week 48	13.891	13.433 - 14.350	<0.001
week 52	14.742	14.284 - 15.201	<0.001

week 56	15.274	14.815 - 15.733	<0.001
week 60	15.827	15.369 - 16.286	<0.001
week 64	16.345	15.878 - 16.813	<0.001
week 68	16.587	15.091 - 17.083	<0.001
week 72	17.474	16.921 - 18.026	<0.001
week 76	17.621	16.976 - 18.266	<0.001
week 80	18.193	17.451 - 19.935	<0.001
week 84	18.034	17.151 - 18.916	<0.001
week 88	17.722	16.361 - 18.814	<0.001
week 92	17.578	16.191 - 18.963	<0.001
week 96	18.202	16.519 - 19.885	<0.001
week 100	17.398	15.039 - 19.757	<0.001
week 104	19.398	17.039 - 21.757	<0.001
week 108	20.262	17.900 - 22.624	<0.001
adlib#week 1	-0.239	-0.891 - 0.416	0.476
adlib#week 2	0.435	-0.222 - 1.092	0.194
adlib#week 3	0.334	-0.311 - 0.981	0.309
adlib#week 4	0.908	0.261 - 1.555	0.006
adlib#week 5	1.123	0.476 - 1.771	0.001
adlib#week 6	1.372	0.725 - 2.020	<0.001
adlib#week 7	1.281	0.635 - 1.927	<0.001
adlib#week 8	1.497	0.849 - 2.144	<0.001
adlib#week 9	1.476	0.829 - 2.124	<0.001
adlib#week 10	1.272	0.625 - 1.919	<0.001
adlib#week 11	1.784	1.136 - 2.431	<0.001
adlib#week 12	1.480	0.836 - 2.124	<0.001
adlib#week 16	1.478	0.829 - 2.127	<0.001
adlib#week 20	0.996	0.349 - 1.644	0.003

adlib#week 24	1.569	0.920 - 2.218	<0.001
adlib#week 28	1.131	0.482 - 1.781	0.001
adlib#week 32	1.006	0.357 - 1.656	0.002
adlib#week 36	1.152	0.503 - 1.801	0.001
adlib#week 40	1.381	0.732 - 2.051	<0.001
adlib#week 44	0.715	0.066 - 1.364	0.031
adlib#week 48	1.348	0.697 - 1.999	<0.001
adlib#week 52	0.747	0.096 - 1.398	0.025
adlib#week 56	0.652	0.001 - 1.303	0.050
adlib#week 60	0.662	0.011 - 1.313	0.046
adlib#week 64	0.464	-0.197 - 1.125	0.169
adlib#week 68	0.805	0.100 - 1.511	0.025
adlib#week 72	0.237	-0.558 - 1.033	0.559
adlib#week 76	0.206	-0.781 - 1.193	0.683
adlib#week 80	-0.861	-2.017 - 0.295	0.144
adlib#week 84	-0.150	-1.650 - 1.350	0.845
adlib#week 88	0.540	-2.062 - 3.142	0.684
adlib#week 92	-0.315	-3.054 - 2.424	0.822
adlib#week 96	0.061	-2.840 - 2.961	0.967
adlib#week 100	1.864	-1.475 - 5.202	0.274
adlib#week 104	-0.136	-3.475 - 3.202	0.936
dam parity	0.355	-0.026 - 0.737	0.068
plasma tp	0.267	0.032 - 0.503	0.026
pneumonia	-0.035	-0.485 - 0.415	0.880
diarrhoea	-0.478	-0.890 - -0.065	0.023
constant	33.454	31.789 - 35.119	<0.001

Random-effects Parameters (variance)	Estimate	95% CI
calf:	0.823	0.611 - 1.109
Bull:	9.96×10^{-18}	1.07×10^{-26} - 9.26×10^{-9}
Residual	1.384	1.311 - 1.461

Table E.16: Multivariable regression model including interaction terms and all variables affecting body condition score during the 0 to 108 week study period. The primary explanatory variable of interest is dietary group (*ad libitum* versus restricted MR). Calf and group are included as random effects.

Outcome variable: bcs	Coefficient	95% CI	P value
ad libitum vs restricted MR	0.085	-0.034 - 0.204	0.160
week 1	-0.165	-0.257 - -0.072	0.001
week 2	-0.279	-0.372 - -0.186	<0.001
week 3	-0.321	-0.412 - -0.229	<0.001
week 4	-0.334	-0.426 - -0.243	<0.001
week 5	-0.189	-0.280 - -0.097	<0.001
week 6	-0.106	-0.197 - -0.016	0.022
week 7	-0.036	-0.126 - 0.054	0.433
week 8	-0.116	-0.206 - -0.026	0.011
week 9	-0.028	-0.118 - 0.061	0.538
week 10	-0.069	-0.158 - 0.021	0.132
week 11	-0.011	-0.101 - 0.078	0.802
week 12	0.028	-0.061 - 0.118	0.537
week 16	0.142	0.052 - 0.232	0.002
week 20	0.064	-0.026 - 0.153	0.164
week 24	-0.011	-0.101 - 0.078	0.802
week 28	0.080	-0.009 - 0.170	0.079
week 32	-0.032	-0.122 - 0.057	0.480
week 36	0.014	-0.076 - 0.103	0.766
week 40	0.070	-0.020 - 0.159	0.126
week 44	0.093	0.003 - 0.182	0.042
week 48	0.129	0.039 - 0.219	0.005
week 52	0.142	0.051 - 0.232	0.002
week 56	0.129	0.039 - 0.219	0.005

week 60	0.172	0.082 - 0.262	<0.001
week 64	0.231	0.140 - 0.323	<0.001
week 68	0.308	0.211 - 0.405	<0.001
week 72	0.296	0.188 - 0.404	<0.001
week 76	0.362	0.236 - 0.487	<0.001
week 80	0.444	0.300 - 0.588	<0.001
week 84	0.540	0.370 - 0.711	<0.001
week 88	0.569	0.358 - 0.780	<0.001
week 92	0.707	0.440 - 0.975	<0.001
week 96	0.805	0.480 - 1.129	<0.001
week 100	1.259	0.805 - 1.714	<0.001
week 104	1.259	0.805 - 1.714	<0.001
week 108	1.577	1.120 - 2.034	<0.001
adlib#week 1	0.052	-0.082 - 0.187	0.447
adlib#week 2	0.155	0.021 - 0.289	0.024
adlib#week 3	0.338	0.206 - 0.471	<0.001
adlib#week 4	0.483	0.350 - 0.616	<0.001
adlib#week 5	0.441	0.309 - 0.573	<0.001
adlib#week 6	0.320	0.188 - 0.451	<0.001
adlib#week 7	0.316	0.185 - 0.446	<0.001
adlib#week 8	0.425	0.295 - 0.556	<0.001
adlib#week 9	0.405	0.274 - 0.535	<0.001
adlib#week 10	0.413	0.282 - 0.543	<0.001
adlib#week 11	0.300	0.170 - 0.431	<0.001
adlib#week 12	0.177	0.047 - 0.307	0.008
adlib#week 16	0.028	-0.103 - 0.159	0.676
adlib#week 20	0.054	-0.077 - 0.184	0.420
adlib#week 24	0.081	-0.050 - 0.212	0.226

adlib#week 28	-0.102	-0.233 - 0.030	0.129
adlib#week 32	0.058	-0.073 - 0.189	0.385
adlib#week 36	0.004	-0.127 - 0.135	0.955
adlib#week 40	0.000	-0.131 - 0.130	0.995
adlib#week 44	0.016	-0.115 - 0.147	0.808
adlib#week 48	0.020	-0.112 - 0.151	0.771
adlib#week 52	0.007	-0.125 - 0.138	0.921
adlib#week 56	0.045	-0.087 - 0.176	0.506
adlib#week 60	-0.002	-0.133 - 0.129	0.974
adlib#week 64	0.044	-0.089 - 0.177	0.517
adlib#week 68	-0.058	-0.199 - 0.083	0.420
adlib#week 72	0.069	-0.089 - 0.227	0.389
adlib#week 76	0.082	-0.112 - 0.276	0.407
adlib#week 80	-0.053	-0.279 - 0.173	0.645
adlib#week 84	0.134	-0.158 - 0.425	0.369
adlib#week 88	0.308	-0.195 - 0.811	0.230
adlib#week 92	-0.030	-0.559 - 0.499	0.910
adlib#week 96	0.072	-0.488 - 0.632	0.800
adlib#week 100	-0.082	-0.726 - 0.562	0.802
adlib#week 104	0.018	-0.626 - 0.662	0.957
dam parity	0.024	-0.040 - 0.087	0.462
plasma tp	0.052	0.013 - 0.091	0.009
pneumonia	-0.074	-0.148 - 0.001	0.054
diarrhoea	0.010	-0.058 - 0.079	0.773
constant	2.415	2.137 - 2.693	<0.001

Random-effects Parameters (variance)	Estimate	95% CI
calf:	0.022	0.016 - 0.030
Bull:	0.009	0.003 - 0.027
Residual	0.051	0.049 - 0.054